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Dedicaces

To my father Moussa and my mother Zoulikha
The reason of my existence in this life and the apple of my eyes

To my brothers Mouhamed, Toufik, Halim and Yacine
and my sisters Noura, Zahra, Dahbia and Rahima
My inspiration and my soul mates

To you who have always encouraged me, you who have trusted me, who have supported me flawlessly in all my projects and who have accepted my choices without necessarily always understanding them, I hope that I will always be up to your expectations

Thank you for making me who I am

With all my love and my gratitude

Your daughter and your sister

Kahina DJAOUD

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Scientific publications

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2. **Djaoud, K.,** Boulekbache-Makhlouf, L., Yahia, M., Mansouri, H., Mansouri, N., Madani, K., Romero, A., 2020a. Dairy dessert processing : Effect of sugar substitution by date syrup and powder on its quality characteristics. *Journal of Food Processing and Preservation* **44**, e14414. https ://doi.org/10.1111/jfpp.14414

3. **Djaoud, K.,** Daglia, M., Sokeng, A.J., Kermiche, F., Arkoub, L., Madani, K., Makhlouf, L.B., 2020b. RP-HPLC-PDA-ESI-MS/MS screening of bioactive compounds from Degla-Beida dates : Conventional and green extraction technologies. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology* **44**(1), 58-81. *https ://doi.org/10.35219/foodtechnology.2020.1.04*

4. **Djaoud, K.,** Boulekbache-Makhlouf, L., Barache, N., Ouinharoun, F., Khodir Madani. Formulation of milk juice based on date and orange juices using the D-Optimal mixture design. *(In drafting phase).*

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Communications

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3. **Djaoud Kahina**, Boulekbache-Makhlouf Lila, Benmeziane Akila, Mouhoubi Khokha, Himed-Idir Hayat and Madani Khodir. Optimisation du processus d'incorporation du sirop et de poudre de dattes communes (*Phoenix dactylifera* L.) dans une crème dessert par le plan de mélange. Séminaire d'Echange National «aux Interface du Développement Durable» SENIDD. December 20-21, 2017, Bejaia, Algeria.
4. **Kahina Djaoud**, Lila Boulekbache-Makhlouf et Khodir Madani. Etude comparative des méthodes d'éxtraction des composés phénoliques à partir des dattes communes algériennes (*Phoenix dactylifera* L.) pour un intérêt de valorisation. Séminaire National pour la Durabilité au Service de notre Alimentation SNDASE. December 20-21, 2017, Bejaia, Algeria.
5. **Kahina Djaoud**, Lila Boulekbache-Makhlouf, Hocine Remini, Myriam Tazarourte, Samir Hadjal et Khodir Madani. Optimisation de l'extraction assistée par micro-ondes du jus de dattes communes (*Phœnix dactylifera* L.) suivie de sa transformation en sirop (Dibs). 1ère Ecole d'hiver sur l'Analyse de cycle de vie et L'eco-conception. February 18–19, 2018, UMBB Boumerdes, Algeria.

6. **Kahina Djaoud**, Lila Boulekbache-Makhlouf, Alberto Romero, Nadia Bouaoudia-Madi and Khodir Madani. Rheological, microstructural and electrophoresis analyses of new dairy desserts prepared with Algerian common date powder and syrup for their valorization. IVème congrès international de biotechnologie et valorisation des bio-ressources. March 21-23, 2018, Tabarka, Tunisia.
7. **Kahina Djaoud**, Lila Boulekbache-Makhlouf, Hocine Remini, Myriam Tazarourte, Samir Hadjal and Khodir Madani. Comparative study ultrasounds and microwaves effects on the yield of total sugars extracted from Algerian common dates : Optimizations using Response Surface Methodology. 29me congrès international des sciences biologiques et de biotechnologie. March 26-29, Sousse, Tunisia.
8. **Kahina Djaoud**, Lila Boulekbache-Makhlouf and Khodir Madani. Participation au salon national des produits de la recherche : Crèmes desserts au sirop et poudre de dattes. July 2-4, 2018, Palais des expositions, SAFEX, Algiers, Algeria.
9. **Kahina Djaoud**, Lila Boulekbache-Makhlouf, Nabila Barache, Ferroudja Ouinharoun, Nassim Hallil and Khodir Madani. Formulation of new milk juice based on date and orange juices using mixture design. October 3-4, 2018, Bejaia, Algeria.
10. **Kahina Djaoud**, Lila Boulekbache-Makhlouf andt Khodir Madani. Evaluation of the antioxidant activity of a common date variety (Degla-Beida) after optimization of the phenolic compounds recovery using microwave-assisted extraction. 4mes journées des Sciences de la Nature et de la Vie. December 15-16, 2018, Bejaia, Algeria.
11. **Kahina Djaoud**, Ferriel Kermiche, Nabila Barache, Ouinharoun Ferroudja, Nassim Hallil, Khodir Madani et Lila Boulekbache-Makhlouf. Monitoring of physicochemical properties of milk juice formulated with date and orange juices during the storage period. Séminaire international sur les produits du terroir : Un outil du développent de l'agriculture de montagne. December 15-16, 2018, Chemini, Bejaia, Algeria.

12. **Kahina Djaoud**, Ferriel Kermiche, Abbou Amina, Imane Announ, Radia Allouache, Khodir Madani and Lila Boulekbache-Makhlouf. Enrichment of common date syrup (*Phoenix dactylifera* L.) with pollen. Séminaire international sur les produits du terroir : Un outil du développent de l'agriculture de montagne. December 15-16, 2018, Chemini, Bejaia.
13. **Kahina Djaoud**, Hocine Remini, Zohra Touati, Myriam Tazarourte, Samir Hadjal, Khodir Madani and Lila Boulekbache-Makhlouf. Valorization of Algerian common dates by the production of date syrup (dibs) using an innovative technique : optimization of ultrasound-assisted extraction of total sugars. Séminaire international sur les produits du terroir : Un outil du développent de l'agriculture de montagne. December 15-16 2018, Chemini, Bejaia, Algeria. (Best poster award)
14. **Kahina Djaoud**, Hocine Remini, Khokha Mouhoubi, Khodir Madani and Lila Boulekbache-Makhlouf. Innovative methods for the preparation of date syrup. Journées doctoriales sur l'innovation et le transfert technologique. July 15-16, 2019, Bejaia.
15. **Kahina Djaoud**, Hocine Remini, Khodir Madani and Lila Boulekbache-Makhlouf. Utilisation du plan de mélange D-Optimal dans l'optimisation de la formulation de crème dessert au sirop et à la poudre de dattes. La 1re journée doctorale des Sciences de la Nature et de la Vie. December 16, 2019, Bejaia, Algeria. (Oral communication).
16. **Kahina Djaoud**, Imane Announ, Radia Allouache, Nassim Hallil, Khodir Madani and Lila Boulekbache-Makhlouf. A processing plan to valorize Degla-Beida dates (*Phoenix dactylifera* L.). La 1ère Conférence nationale Analyse : de cycle de vie, Eco-Conception, Eco-éducation, Eco-innovation au service du développement durable. May 13-14, 2020, Boumerdes, Algeria.

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List of abreviations

AAE	Ascorbic acid equivalent ;
ABTS	2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ;
ANOVA	Analysis of variance ;
BBD	Box Behnken Design ;
CDD	Commercial dairy desssert ;
CE	Conventionnel extraction ;
CFU	Colony Forming Unit ;
CLSM	Confocal laser scaning microscopy ;
DP	Date powder ;
DPPH	2,2-diphenyl-1-picrylhydrazyl ;
DS	Date syrup
DW	Dry weight ;
EtOH	Ethanol ;
ESI	Electro Spray Ionization ;
GAE	Gallic acid equivalent ;
GE	Glucose equivalent
HPLC-RID	High performance liquid chromatography-refractive index detector ;
Htz	Hertz ;
JMP	John's machintosh project ;
JORA	Official journal of the Algerian republic ;
MAE	Microwave-assisted extraction ;
MDD	Mixture dairy desssert ;
MeOH	Methanol ;
MS	Mass spectrometry ;
m/z	Mass/charge ;
PDD	Powder dairy desssert ;
pH	Hydrogen potential ;
QE	Quercitin equivalent ;
$\mathbf{R^2}$	Correlation coefficient ;
$\mathbf{R^2_{adj}}$	Adjusted correlation coefficient ;

RMSE	Root mean squar error ;
rpm	Rotation per minute ;
RSM	Response surface methodology ;
SDD	Syrup dairy desssert ;
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis ;
SSC	Solid soluble content ;
TPC	Total phenolic content ;
TSC	Total sugar content ;
UAE	Ultrasound-assisted extraction ;
UV-Vis	Ultraviolet-visible ;
v/v	Volume/volume.

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General introduction

General introduction

Date palm fruit (*Phoenix dactylifera* L.), an endemic functional food with great nutritional and economic importance due to its phytochemical composition (Al Alawi et al., 2020), has been consumed for millennia and has long been rooted in Berber/Amazigh and Arabic cultures (Gros-Balthazard et al., 2020). It is an ideal substrate for deriving a range of added value products in food and nutraceutical industries in the coming future, employing bioprocessing technologies, which have immense scope for application in the valorization of date by-products (Younas et al., 2020). The valorization of organic wastes is an important strategy for human and environment safeguard, reducing agricultural costs and chemical products use (Abid et al., 2020). The annual worldwide production of date fruits was 8.53 million tons, placing Algeria in the 4th place of date producing countries with 1.10 milion tons (FAOSTAT, 2020). The annual date fruit wastes for these countries amount to 0.84 million tons. These huge quantities, provide ample scope for the emergence of new bioindustries, towards a total utilization of the fruit, in addition to efficient and effective waste management (El-Mously et al., 2019).

Many authors have investigated the nutritional composition of different date fruit varieties "a medicinal food" cultivated in different parts of the world (Abdulrahman et al., 2020; Al Juhaimi et al., 2020; Kuras et al., 2020). The main nutrients that date fruit provides are carbohydrates (44–88%), which gives energy swiftly, the content depends on the variety and stage of ripening. The high nutritional value of dates is also associated with the dietary fiber content (6.40–11.50%) (Qadir et al., 2020). In addition, to accelerate intestinal motility, it also affects the reduction of sugar and total cholesterol levels in the blood (Kuras et al., 2020). Moreover, date fruit contain fats (0.20–0.50%), proteins (2.30–5.60%), minerals (0.10–9.16 mg/100 g DW), vitamins (A, B and E) (Qadir et al., 2020) and bioactive compounds (Maqsood et al., 2020).

The lost dates, commonly named “by-products”, are not consumed by humans because of their inadequate texture, contamination with fungus as well as infestation by insects or simply due to their low quality (El-Mously, 2019). These by-products can be processed into different products including date syrup (Derouich et al., 2020). Date syrup called “dibs or molasses” is probably the most common derived date product. It can be directly consumed or used as a natural sweetener and flavoring for human consumption in some food formulations such as ice cream, confectionery and jam (Gheisari et al., 2020; Khapre and Shah, 2016; Mahmed et al., 2019), as well as beverages (Benidir et al., 2020), yogurt (Arfaoui, 2020) and dairy dessert (Jridi et al., 2015).

The global health market has been invaded by functional food (da Silva et al., 2019), identified as one of the leading food categories, where research and development efforts are concentrated. Hence, major companies have become more curious and eventually ventured in the development of food for health and wellness market (Dable-Tupas et al., 2020). Consumers show an increasing demand for healthier food products, desirably free of chemical additives (Sayas-Barberá et al., 2020). Recent studies have been conducted dealing with the effect of alternative sweeteners to sucrose on the rheological behaviour, sensory and physicochemical properties, and identifying several beneficial attributes of dairy desserts (Gurditta et al., 2019; Jahromi and Niakousari, 2018; Prasad et al., 2018). However, the available studies only evaluate the impact of this substitution on the characteristics of the products. In this sense, the manufacturing process of dairy desserts is not studied in an optimization point of view. The incorporation of date syrup and powder in the formulation of dairy desserts could be an alternative for the valorization of industrial by-products as well as by the manufacture of health and functional food products with high added values (Jridi et al., 2015).

The food industry is seeking to improve system performance and to enhance process efficiency without increasing cost and time. Finding a condition with the best output for a system is the main purpose of the optimization aproach, which has been commonly used (Sevda and Singh, 2020). In the past, the optimization in food processes has been performed through investigating the effect of one-parameter changes on a response while all the others are held at a constant level (Kilic et al., 2019).

The main disadvantages of this method are the interactive effects among the variables, which are not considered and there is a lack of explanation of the complete effect of the factors on the response. In addition, this method increases the number of experiments needed to conduct the research, which leads to increase cost and time (Sevda and Singh, 2020; Kilic et al., 2019). In order to solve this problem, optimization studies can be carried out by using multivariate statistic methods (Sevda and Singh, 2020). The response surface methodology (RSM) is the most popular multivariate statistic technique, which has been used in the optimization of food processes (Sevda and Singh, 2020). It is a collection of statistical and mathematical methods established on the fit of a polynomial model to the data that must depict the behavior of a data set with the purpose of making statistical predictions. The approach is useful for optimizing, designing, developing and improving processes where a response or responses are affected by several variables (Ghorbannezhad et al., 2016).

In Algeria, low quality dates especially dry varieties, representing more than 30% of the national production, are not valorized enough and destined generally for animals feeding. The high concentration of biogenic compounds and the intrinsic nutritional features of date fruits make it a suitable raw matrix for manufacturing of functional ingredient dietary supplements or pharmaceutical preparations (Di Cagno et al., 2017). To our knowledge, this is the first study reporting the use of optimized date syrup and powder from common date fruits for manufacturing of dairy desserts, as a sugar substitute or a dietary supplement with health promoting features. This thesis is divided into two parts, the first is a bibliographic synthesis, with two chapters, giving an overview on the date palm, date fruit and date by-products. A simple and easily understandable introduction to the experimental design, theoretical principles of the response surface methodology and steps for its application, and optimization process were also described to introduce readers to this multivariate statistical technique.

The practical part of the present work was divided into three complementary sections. The first, investigates the dry date fruit from Degla-Beida variety growing in Algeria, with regard to their morphological, physicochimical and nutritional properties by the characterization of its phytochimical compounds using the RP-HPLC-ESI-MS/MS.

In the second section, the optimization of date syrup production by microwave-assisted, ultrasound-assisted and water bath extraction methods, using the response surface methodology (RSM) with Box-Behnken design (BBD) was carried out. The third objective of this study was to optimize the formulation of new dairy desserts with date syrup and powder as an alternative sweetener-ingredients, using the D-optimal mixture design, in order to substitute sugars. As the formulation of new food products influence the final product quality, we have investigated the possible influence of this incorporation on the sensory, physicochemical, phytochemical, microbiological, rheological and microstructural characteristics of the dairy desserts: syrup dairy dessert (SDD) and optimal formulation of mixture dairy dessert (MDD), the results were compared with a commercial dairy dessert (CDD).

Part I

Bibliographic Part

Chapter 1

Date palm fruit

1.1 Date palm

1.1.1 Origin

Date palm (*Phoenix dactylifera* L.) is considered as one of the oldest fruit tree domesticated by man and mentioned in the Qur'an "Shake the trunk of the palm tree towards thee: it will drop fresh, ripe dates upon thee. Eat then drink, and let thine eye be gladdened." (Qur'an 19: 25–26) (Mahmud et al., 2019). Date palm is cultivated in subtropical and tropical areas since the ancient era for its edible sweet fruits known as dates. Although its exact place of origin is still uncertain, it is assumed that it originated from a region straddling between Egypt and Western Asia (Awad et al., 2020; Sallon et al., 2020). The cultivation of the tree is dated back to 5500–3000 B.C. It is currently and mainly cultivated in the semi-arid and desert areas of the Middle East, Iraq, Iran, Oman, Pakistan, India, in the Canary Islands, and in the northern African countries such as Algeria, Egypt, Tunisia and Morocco (Alshwyeh, 2020; Deshpande and Deshpande, 2017). Date palm are also grown in Australia, Mexico and the United States, especially in Southern California, Arizona and Texas (Aljaloud et al., 2020).

1.1.2 Botanical description and vernacular names

Tall tree up to 36 m in height, occasionally found cultivated or self grown (Figure 1.1). Trunks covered with persistant bases of petioles, the base usually surrounded by a mass of offshoots or sudcen; leaves in poen crown, pinnate 20–40 cm long, linear, keeled lower pinnae modified into spines; flowers in branched spadices, small; fruit an oblong berry 2.5–7.5 cm long, reddish or yellowish brown when ripe; seed cylindric, hard with a longitudinal furrow (Deshpande and Deshpande, 2017). The date palm belongs to the Plantae kingdom, Tracheobionta subkingdom, Spermatophyta superdivision, Magnoliophyta division, Liliopsida class, Arecidae subclass, Arecales order, Arecaceae family, Phoenix genus and dactylifera species (Ghnimi et al., 2017).

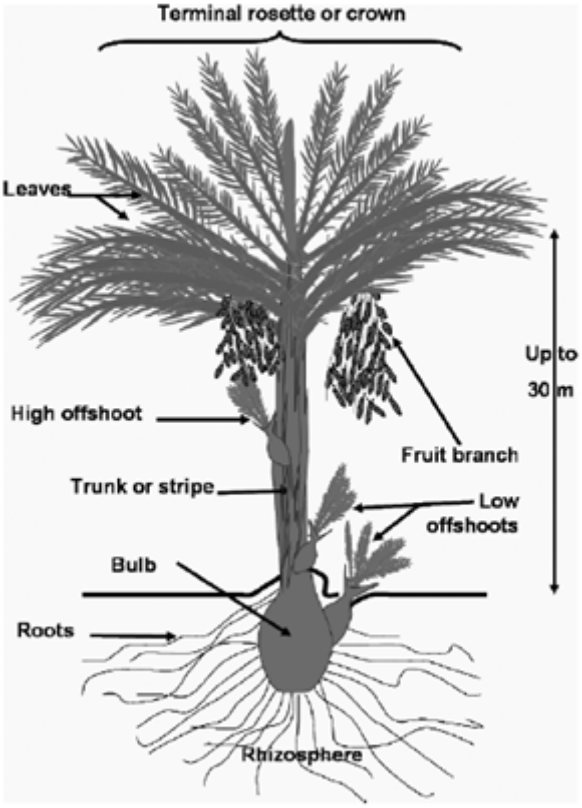


Figure 1.1. Represention of the vegetative clothing of date palm (Awad et al., 2020).

Some vernacular names of date palm are highlighted as described by Deshpande and Deshpande (2017): Arabic–Nakhleh, Greek–Phoenix, French–Dattier, English–Date palm, Spanish–Datiler, Italian–Date, German–Daten, Portugese–Datas, Bengali–Khejur, Dutch–Datum, Gujrati–Khajur, Hebrew–Tamar, Hindi–Khajur, Malayalam–Pranthapuzam, Marathi–Khajur, Oriya–Khejuri, Punjabi–Pindakhajur, Russian–Finik, Sanskrit–Kharjur, Urdu–Khurma, Telugu–Khajurpupandu, Tamil–Perichampazham.

1.1.3 Geographic distribution

Date palm tree count for more than 120 million trees across the world and cover an area of around 250 000 ha. Each tree grows for more than 100 years, producing fruits and waste residues on annual harvests (Awad et al., 2020). They are distributed on all continents and cultivated in many countries including "Middle East, North Africa, Central and South America, Southern Europe, Iraq, Iran, Saudi Arabia, Algeria, Egypt, Tunisia, Libya, Morocco, Sudan, Oman and some parts of India and Pakistan" (Qadir et al., 2020).

Date palm is grown in numerous oases spread over the southern part of Algeria, where the climate is hot and dry. Given the geography of the country, it is possible to describe several regions of their cultivation as described by Bouguedoura et al. (2015):

- In the Atlas Mountains foothills (Ksour Ouled Naïl, Zibans and Aures), there is an oasis chain that marks the gateway of the Sahara.
- In the east, Zibans (Biskra), Oued Righ, Oued Souf (El Oued) and the basin of Ouargla especially with Deglet Noor cultivar of high commercial value.
- In the west, Saoura (Beni Abbes), Touat (Adrar), Gourara (Timimoun) and Tidikelt (Reggane) where palm groves include cultivars of relatively low commercial quality. It is in this area where the only truly bayoud-resistant cultivar, Taqerbucht, exists.
- At the center, El Golea, the M'zab (Ghardaïa) and Laghouat .

1.2 Date fruit

1.2.1 Description

Date fruit is composed of a pericarp, mesocarp, endocarp and one seed (also called kernel, pit, or pyrene). The mesocarp, representing the most part of the pulp, consists of enlarged parenchymatous cells and is divided into outer-mesocarp and inner-mesocarp intermediated by 3–10 layers of tanninferous cells. The seed has a ventral side characterized by a furrow of varying depth and width running along its length. The dorsal side of the seed is convex with a small shallow hole called the micropyle under which lies the embryo. Seeds from different date varieties differ in the depth of the furrow and the position of the micropyle (central or peripheral) (Ghnimi et al., 2017).

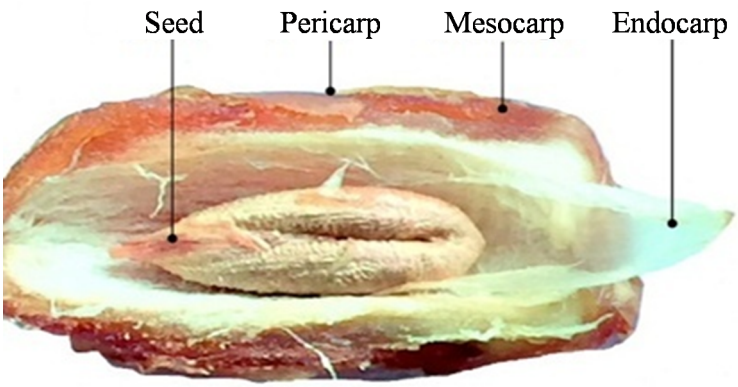


Figure 1.2. Anatomy of the date fruit showing the pericarp, mesocarp, endocarp and seed (Hussain et al., 2020).

1.2.2 Ripening behavior

Nutritional and phytochemical properties of date fruit vary depending upon the harvest stage, variety and preparation treatment (Hussain et al., 2020). The fruit development stages of dates can be described as follows:

1. **Hababouk stage:** It is the initial stage that starts after fertilization and lasts for 4–5 weeks, with 80–90% moisture.

2. **Kimri stage:** During this stage, the fruit increases in length, weight, sugar levels, and acidity. This stage ends with a change in fruit color to yellow or red (depending upon the variety involved).

3. **Khalal stage:** At this stage, the fruit starts to turn from green to yellow purplish-pink, red or yellow scarlet (depending on variety). The glucose content increases while the moisture content decreases up to 50%. The tannins start to precipitate and lose their astringency, making the dates more palatable.

4. **Rutab stage:** Sucrose changes into invert sugars with less tannin than the previous stage and moisture contents decreases to 35–40%. The dates become soft, half-ripe and turn light brown in color.

5. **Tamar stage:** At this stage, invert becomes the predominant sugar in the fruit. The dates become soft with 20–25% moisture contents.

1.2.3 Classification and varieties

Date fruit shape varies from oval to cylindrical, with dimensions from 3 to 11 cm in long and 2 to 3 cm in diameter. However, the color varies from yellow, brown, red, to black. The fruits are also different in the hardness of the edible part and are classified according to their moisture contents at fresh Tamar stage into soft (more than 30% moisture), semi-dry (between 20–30% moisture) and dry varieties (less than 20% moisture) (Idowu et al., 2020). Generally, soft date cultivars were dominated by invert sugars (fructose and glucose) and contain little or no sucrose while dry varieties may contain a relatively high proportion of sucrose. According to sugar type, date varieties were classified into **(i)** invert sugar varieties containing mainly glucose, **(ii)** mixed sugar varieties and **(iii)** cane sugar varieties containing sucrose as the main sugar. There is some agreement and disparity in the classification of date varieties according to moisture content or sugar type suggesting that the relationship is not strictly linear possible due to interference from fiber content and type (Ghnimi et al., 2017).

The world's date palm biodiversity accounts to 5 000 date palm cultivars based on the botanical descriptions done by several researchers. About 1 000 cultivars can be found in Algeria, 400 in Sudan, 370 in Iraq, 250 in Tunisia, 244 in Morocco, 400 in Iran, 196 in the USA and 26 in Egypt, as well as plenty more cultivars in other states (Awad et al., 2020). Some popular varieties are Ajwa, Abel, Al-Barakawi, Assel, Bireir, Barhi, Bamy, Dabbas, Dayri, Deglet-Noor, Dhakki, Dora, Degla, Ftimi, Hilali, Hallawi, Khu-

naizi, Khalas, Khodri, Khadrawy, Kentichi, Lasht, Munifi, Medjool, Ruthana, Ruchdi, Sukkary, Sefri, Segae, Thoory and Zahidi, (Deshpande and Deshpande, 2017).

1.2.4 Biochemical composition

This paragraph highlights on the nutritional and antioxidant constituents of date fruits (Table 1.1). Dates are a rich source of carbohydrates and dietary fibers with contents up to 88.02 and 10.95%, respectively. However, they are low in proteins and fats, less than 3.30 and 0.56%, respectively. The presence of minerals is also noteworthy in dates at up to 0.62% in some varieties. They are a considerable source of bioactive molecules, in particular carotenoids and phenolic compounds that can prevent and/or reduce the risk of diseases due to oxidative stress (Benmeziane-Derradji, 2019).

1.2.5 Pharmacology and bioactivities

Since long, both fruits and stones of *Phoenix dactylifera* L. have been used in different systems of traditional medicine. It has been found that dates were traditionally used in the treatment of diabetes and hypertension (Obode et al., 2020; Saddi et al., 2018). Recent studies explained the benefits of date palm fruit on labor process improvement. They can be considered as a factor for reducing the frequency of caesarean section in order to prevent its great complications. Date fruit due to energy production and having enough calories, can be helpful for pregnant women during labor, and it can also prevent physical weakness. Furthermore, because it contains simple sugars, it is fast in digestion and absorption (Bagherzadeh Karimi et al., 2020).

1.2.6 Production, statistics and economics

Date palm is a strategic fruit crop, the worldwide production was 8 526 218 tons (FAOSTAT, 2020). Algeria is one of the largest date producers in the world (Bedjaoui and Benbouza, 2020), with 1 094 700 tons harvested in 2018, it ranks the fourth worldwide (FAOSTAT, 2020). Date production in Algeria varies annually, which correlated with the alternate bearing of date palms, cultural practices, climatic hazards and region of cultivation (Bouguedoura et al., 2015).

Table 1.1. Biochemical composition of date fruit (Al-Farsi and Lee, 2008).

Composition	**Low level**	**High level**
Water content (g/100 g)	7.2	50.4
Ashes (g/100 g)	1.0	1.9
Lipids (g/100 g)	0.1	1.4
Proteins (g/100 g)	1.1	2.6
Amino acids (mg/100 g)		
Alanine	30	133
Arginine	34	148
Aspartic acid	59	309
Cysteine	13	67
Glutamic acid	100	382
Glycine	42	268
Histidine	0.1	46
Isoleucine	4	55
Leucine	41	242
Lysine	42	154
Methionine	4	62
Phenylalanine	25	67
Proline	36	148
Serine	29	128
Threonine	23	95
Tryptophan	7	92
Tyrosine	15	156
Carbohydrates (g/100 g)	52.6	88.6
Fructose	13.6	36.8
Glucose	17.6	41.4
Sucrose	0.5	33.9
Fibers (g/100 g)		
Soluble	0.4	1.3
Insoluble	3.03	7.4
Total	3.57	10.9
Minerals (mg/100 g)		
Mg	31.0	150
Na	1.00	261
Ca	5.00	206
P	35.0	74
K	345.0	1287
Mn	0.01	0.4
Fe	0.10	1.5
Zn	0.02	0.6
Cu	0.01	0.8
Se	0.24	0.4
Vitamins (μg/100 g)		
A (Retinol)	3.0	44.7
B_1 (Thiamine)	50	120
B_2 (Riboflavin)	60	160
B_3 (Niacin)	1274	1610
B_6 (Pyridoxal)	165	249
B_9 (Folate)	39	65
C (Ascorbic acid)	4	16
α-Carotenoids	3	3
β-Carotenoids	2.5	146
Zeaxanthin	33	33.0
β-Zeaxanthin	9	9.0
Lutein	28	541
Neoxanthine	184	381
Phenolic acids (mg/100 g)	3.91	661
Anthocyanins (mg/100 g)	0.2	1.5

Statistics show that date production in Algeria is mainly concentrated in the southeastern part of the country, which is responsible of 76% of national production. The province of Biskra ranks first with nearly 31% followed by El Oued 27% and Ouargla with 18%. The edaphic and pedoclimatic specificities, as well as the crop management and the market value of cultivars, justify the importance of the production in these regions. In date groves elsewhere, production is less important, contributing 24% of total national production allocated as follows: southwest (15%) and south center (9%) (Bouguedoura et al., 2015). Approximately 18 million date palms are cultivated on an area of 169 380 ha; out of these, ten million trees are producing an annual yield of 500 000 mt of dates. Exports of Algerian dates are small because of weak marketing strategies. A program for the development and expansion of date palm agriculture was implemented by the Ministry of Agriculture and Rural Development in the recent years. Moreover, research on the propagation, improvement, and evaluation of Algerian date palm cultivars is receiving attention by researchers in various universities and research institutes (Bouguedoura et al., 2015).

1.2.7 Date by-products

Phoenix dactylifera L. are frequently used to prepare a wide range of products such as folklore medicines, date fruit juice concentrates (spread, syrup and liquid sugar), fermented date products (wine, alcohol, vinegar and organic acids), and date pastes for different uses (*e.g.* bakery and confectionary) besides their direct consumption (Rahmani et al., 2014). Date palm industries produce a variety of products including "date paste, date syrup, date dip, date honey, date jam and date vinegar". Some of the products including "date pectin, dietary fiber and date syrup" are used as gelling agents in food industries (Di Cagno et al., 2017). The incorporation of date palm seed waste extract has been employed effectively and confirmed as capping material which stabilized zinc-oxide nanoparticles (El-Naggar et al., 2018). The vesicular structure of the optimized date seed oil loaded nano-vesicles with nano-size range and good stability features were confirmed in most recent study. Date seed oil loading in niosomes revealed a significant enhancement toward inflammation alleviation, which offers a promising implement in osteoarthritis remediation and prohibition (Soliman et al., 2018).

However, date syrup has been investigated in the preparation of foodstuffs including "jams, marmalades, concentrated beverages, chocolates, ice cream, confectioneries, sweets, snacks, bakery products and healthy food" (Di Cagno et al., 2017). Macroporous natural sporopollenin exine capsules were obtained from *Phoenix dactylifera* L. and coated with carboxymethyl cellulose and epichlorohydrin. The polymer coated capsules were applied in controlled delivery of model drug paracetamol. The results suggested enhanced release of paracetamol in phosphate buffer solution in comparison with simulated gastric fluid (Alshehri et al., 2016). In the modern era, people are becoming more conscious about their outlook especially skin and they seek natural alternatives to replace hazardous chemical cosmetics available in the market. Therefore, the use of date seed oil has been increased dramatically for the care and protection of skin, as it is comprised of essential fatty acids that our body cannot synthesize like linoleic acid (Adeosun et al., 2016).

1.2.8 Utilization in bio-processing technology

The bioprocessing technology is the application of microorganisms in "submerged fermentation, solid state fermentation or slurry state fermentation" under immobilized condition and use of enzymes for biotransformations of organic materials into desired biomass production (Di Cagno et al., 2017; Khan et al., 2018). The tons of date fruit wastes are discarded daily by the industries which creates environmental problems (Di Cagno et al., 2017). Thus, it is essential to utilize wasted date seeds for appropriate applications. Dates are a good source of sugar and hence it can be utilized as a raw material in fermentation process. This utilization is relatively cheaper and does not require any special treatment such as "acid hydrolysis, steam explosion, or enzymatic treatment". The waste products of date fruit have greater application in bioprocess technologies to produce several value added products (Qadir et al., 2020). The health prospects of these processing "by-products and wastes" are helpful in the production of biosurfactants, biopolymers, antibiotics, biofuels, industrial enzymes, organic acids, and other promising industrial molecules need to be thoroughly examined (Di Cagno et al., 2017). Yahmed et al. (2020) demonstrated the feasibility of using discarded dates in a processing approach aimed at producing biofuels namely biohydrogen and biogas while recovering the residual soluble sugars (date syrup) as high added value product.

1.2.9 Situation of date processing in Algeria

1.2.9.1 Artisanal processing

Traditional transformation of dates is very popular and practiced by local population. The expansion of this craft activity would contribute to the stimulation of new markets, and consequently the extension of the date palm sector. Knowledge of the characteristics of existing varieties to consider better development. Many products are made from dates for different uses: food (cakes, honey, flour, juice, jam...), pharmacopoeia (various treatments) and cosmetics (eye shadow, masks, khol for eyes ...). These products, which date back to antiquity, are still saved, developed and improved (Haffas, 2006).

1.2.9.2 Industrial transformation

Despite numerous research works on the transformation of dates and palm by-products, in the current state of things there is no industrial transformation unit in production for all the work that has been done previously in some Algerian Universities and research structures across Algeria. Which fall within the framework of the transformation and the valorization of non-marketable dates or of low market value, as raw material for the production of other products with high added value (Haffas, 2006).

1.2.10 Date syrup as a sucrose substitute

Date syrup locally known as “dibs” is probably the most common derived date fruits (Al-Khayri et al., 2015). It is a natural extract of dates, without any additives, colors or preservatives (Doma et al., 2013). Date syrup contains various components such as carbohydrates, especially glucose and fructose, proteins, lipids, pectin, salts and minerals including potassium, calcium, magnesium and iron as well as organic acids such as malic, succinic, acetic, citric and oxalic acids (Derouich et al., 2020). Date syrup is a rich source of phenolic compounds which are known potent radical scavengers, and also it has antibacterial activity attributed to its bioactive components including plant-derived phenolic molecules (Taleb et al., 2016). Since it is a good source of nutritious and bioactive compounds, it can potentially be consumed directly or used as a sweetening alternative (sucrose substitute) in food formulations (Nasrolah et al., 2019).

Dairy food industries look to develop new milk-based products to meet consumer demand by addressing health concerns through fortification and combining added-value ingredients (Cadena et al., 2012). Many researchers reached the conclusion that food high in added sugars have an adverse effect on diet quality and dietary references intake, especially for children (Durward, 2020; Erickson and Carr, 2020; Kumar et al., 2020). Date syrup as a natural and nutritional additive is one of the best choices for dairy products flavoring and a safe alternative to sugars (Keshtkaran et al., 2013).

As a product category, dairy desserts have become increasingly popular. The volume of ready-to-eat (RTE) dairy desserts consumed globally has grown rapidly, with a proliferation of types and variants. This growth has been prompted by several consumer drivers, including convenience, nutrition, and sensory appeal. There is an interesting dichotomy in this food category indulgence and health foods. In fact, dairy desserts represent some of the best examples of true healthy indulgence in a food product. In all regions of the world, chocolate is the number one flavor in dairy desserts, with other popular flavors being strawberry, vanilla and caramel. Different fruit flavors are starting to feature more prominently (Saunders, 2011).

The role of excessive sugar intake on health and disease is currently an active area of scientific and debate. The use of date syrup is not common approach in dairy science, to our knowledge, researchers have not treated this issue in much detail. So, little is published on the functional properties of different concentrations of date syrup in dairy products. It is recommended that further research be undertaken in this field. All dairy formats provide excellent bases for fortification with additional vitamins, minerals and bioactives. While the traditionally indulgent dessert categories have not been obvious targets for health-promoting additives, this situation is changing, as consumers seek health benefits throughout the food product range (Azaïs-Braesco et al., 2017).

Chapter 2

Overview of some theoretical concepts on experimental designs

2.1 Response surface methodology (RSM)

Traditionally, optimization in analytical chemistry has been carried out by monitoring the influence of one factor at a time on an experimental response. While only one parameter is changed, others are kept at a constant level. This optimization technique is called one-variable-at-a-time. Its major disadvantage is that it does not include the interactive effects among the variables studied. As a consequence, this technique does not depict the complete effects of the parameter on the response (Abou-Taleb and Galal, 2018). Another disadvantage of the one-factor optimization is the increase in the number of experiments necessary to conduct the research, which leads to an increase of time and expenses as well as an increase in the consumption of reagents and materials. In order to overcome this problem, the optimization of analytical procedures has been carried out by using multivariate statistic techniques (Bezerra et al., 2008). Among the most relevant multivariate techniques used in analytical optimization is the response surface methodology (RSM). The RSM is a tool that contributes to process optimization, and highlights the most efficient conditions and possible interactions between experimental variables (Oludemi et al., 2018; Pandey and Kaur, 2018).

The response surface methodology is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the experimental data, which must describe the behavior of a data set with the objective of making statistical previsions (Bezerra et al., 2008). It was developed by Box and Wilson in 1951 to help the improvement of manufacturing processes in the chemical industry (Dean et al., 2017). This term was originated from the graphical perspective generated after fitness of the mathematical model, and its use has been widely adopted in texts on chemometrics. Toward this objective, linear or square polynomial functions are employed to describe the system studied and, consequently, to explore (modeling and displacing) experimental conditions until its optimization (Sudha et al., 2017).

2.1.1 Terminology

Before beginning the discussion on the application of the RSM in the optimization of analytical methods, it is pertinent to introduce some key terms (Dean et al., 2017).

Experimental domain is the experimental field that must be investigated. It is defined by the minimum and maximum limits of the experimental variables studied.

Experimental design is a specific set of experiments defined by a matrix composed by the different level combinations of the variables studied. Doehlert is an example of a second-order experimental design. This design defines a specific set of combinations for the levels of variables that must be applied experimentally to obtain the responses.

Factors or independent variables are experimental variables that can be changed independently of each other. Typical independent variables comprise the pH, temperature, reagents concentration, microwave irradiation time, flow rate, atomization temperature and elution strength, among others.

Levels of a variable are different values of a variable at which the experiments must be carried out. The variable pH, for example, can be investigated at five levels: 4, 5, 6, 7 and 8 in the optimization of a spectrophotometric method.

Responses or dependent variables are the measured values of the results from experiments. Typical responses are the analytical signal (absorbance, net emission intensity and electrical signal), recovery of an analyte, resolution among chromatographic peaks, percentage of residual carbon and final acidity, among others.

Residual is the difference between the calculated and experimental result for a determinate set of conditions. A good mathematical model fitted to experimental data must present low residuals values.

2.1.2 Steps for RSM application

Some application steps of the response surface methodology (RSM) as an optimization technique, according to Bezerra et al. (2008), are described as follows: **(1)** selection of independent variables of major effect on the system through a preliminary study, and delimitation of the experimental region, according to the objective of the study and the experience of the researcher; **(2)** choice of the experimental design and carrying out the experiments according to the selected experimental matrix; **(3)** mathematical–statistical treatment of the obtained experimental data through the fit of a polynomial function; **(4)** evaluation of the model's fitness; **(5)** verification of the necessity and possibility of performing a displacement in direction to the optimal region; and **(6)** obtaining the optimum values for each variable.

2.1.2.1 Preliminary study

A wide range of variables may affect the response of the studied system, and it is practically impossible to identify and control the small contributions from each one, due to high cost and number of runs needed. Therefore, it is necessary to select those variables with major effects. A preliminary study, the first step of the optimization process, should be carried out to determine which of the several experimental variables and their interactions present more significant effects.

2.1.2.2 Choice of the experimental design

The simplest model which can be used is based on a linear function. For its application, it is necessary that the obtained responses are well fitted to the following equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i \, x_i + \varepsilon \tag{2.1}$$

Where k is the number of variables, β_0 is the constant term, β_i represents the coefficients of the linear parameters, x_i represents the variables, and ε is the residual associated to the experiments.

Therefore, the responses should not present any curvature. To evaluate curvature, a second-order model must be used. Two-level factorial designs are used in the estimation of first-order effects, but they fail when additional effects, such as second-order effects, are significant. So, a central point in two-level factorial designs can be used for evaluating curvature. The next level of the polynomial model should contain additional terms, which describe the interaction between different experimental variables. This way, a model for a second-order interaction presents the following terms:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i \, x_i + \sum_{1 \leq i \leq j}^{k} \beta_{ij} \, x_i \, x_j + \varepsilon \tag{2.2}$$

Where β_{ij} represents the coefficients of the interaction parameters. In order to determine a critical point (maximum or minimum), it is necessary for the polynomial function to contain quadratic terms according to the equation presented below:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i \, x_i + \sum_{i=1}^{k} \beta_{ii} \, x_i^2 + \sum_{1 \leq i \leq j}^{k} \beta_{ij} \, x_i \, x_j + \varepsilon \tag{2.3}$$

Where β_{ii} represents the coefficients of the quadratic parameter.

To estimate the parameters in equation (2.3), the experimental design has to assure that all studied variables are carried out at in at least three factor levels. Thus, two modeling, symmetrical response surface designs are available. Among the more known second order symmetrical designs are the three-level factorial design, Box–Behnken design, central composite design, and Doehlert design. These symmetrical designs differ from one another with respect to their selection of experimental points, number of levels for variables, and number of runs and blocks.

After screening for the important factors in the preliminary study and the choice of the experimental design, the levels of factors are defined. The codification of the variable levels consists of transforming each studied real value into coordinates inside a scale with dimensionless values (-1 to +1), which must be proportional at its localization in the experimental space. The codification is of concern because it enables the investigation of variables of different orders of magnitude without the greater influencing the evaluation of the lesser.

2.1.2.3 Mathematical–statistical treatment of data

After acquiring data related to each experimental point of a chosen design, it is necessary to fit a mathematical equation to describe the behavior of the response according to the levels of values studied.

2.1.2.4 Verification of the fitted model

The mathematical model found after fitting the function to the data can sometimes not satisfactorily describe the studied experimental domain. The more reliable way to evaluate the model fitted quality is by the application of analysis of variance (ANOVA). The central idea of ANOVA is to compare the variation due to the treatment (change in the combination of variable levels) with the variation due to random errors inherent to the measurements of the generated responses. From this comparison, it is possible to evaluate the significance of the regression used to foresee responses considering the sources of experimental variance. Several techniques are available, such as prediction error, sum of squares, residuals and the lack-of-fit test. The coefficient of determination (R^2) is used to evaluate the general predictive capability of the fitted model.

2.1.2.5 Determination of the optimal conditions

The response surfaces generated by linear models can be used to indicate the direction in which the original design must be displaced in order to attain the optimal conditions. However, if the experimental region cannot be displaced due to physical or instrumental reasons, the research must find the best operational condition inside the studied experimental condition by visual inspection. For quadratic models, the critical point can be characterized as maximum, minimum or saddle. It is possible to calculate the coordinates of the critical point through the first derivate of the mathematical function, which describes the response surface and equates it to zero. The visualization of the predicted model equation can be obtained by the surface response plot. This graphical representation is an n-dimensional surface in the (n+1)-dimensional space. Usually, a two-dimensional representation of a three-dimensional plot can be drawn. Thus, if there are three or more variables, the plot visualization is possible only if one or more variables are set to a constant value.

2.1.3 Experimental designs

The response surface methodology (RSM) establish a quadratic models, they allow the obtainment of the factors critical conditions. The most employed designs in food industry are: Box-Behnken designs (BBD) and mixture design (Beg, 2021; David and Mahanty, 2021; Rohilla and Mahanta, 2021; Sumampouw et al., 2021).

2.1.3.1 Box Behnken design (BBD)

Box Behnken matrix for three factors is a spherical, rotatable design, which viewed on a cube, it consists of the central and middle points of the edges (Ferreira, 2015). In this design, all the factors are studied with three levels, and the number of experiments required for obtaining the model is defined as $N = 2k(k-1) + C_0$, where k is the number of factors and C_0 is the number of replications in the central points (Malekjani and Jafari, 2020). As a type of response surface methodology, the Box-Behnken design (BBD), compared with the central composite design (CCD) and design of experiment (DOE), has a higher efficiency, less-extreme experimental conditions and more reliable data (Ma et al., 2020).

2.1.3.2 Mixture design

The mixture design is also a response surface methodology, which the product resultant is an ingredient mixture, whose the proportion of the components is established by models which may be linear, quadratic or cubic (Handa et al., 2016; Sifaoui et al., 2016). The applications of mixture designs in food analysis are still sparse, we can cite some applications of mixture designs for the optimization of gluten-free sponge cake fortified with whey protein concentrate (Ammar et al., 2020), optimization of maize–millet based soy fortified composite flour (Sahu and Patel, 2020) and optimization of juice cocktail containing soursop, pineapple, orange and mango (Akonor, 2020).

The construction of the mixture design is very reduced in time, to a theoretical mixture of three components A, B and C called factors which are at the top of an equilateral triangle. This technique differs from the ordinary response surface where the sum of all the components A, B and C must be equal to 100% (Bezerra et al., 2020) (Equation 2.4). If it is expressed in units, this sum will be equal to 1. In this case, each component x_1, which represents the proportion of its ingredients in a mixture, is between two levels 0 and 1. We can express this sum by the following equation which is named (fundamental constraint of mixtures).

$$\sum_{i=1}^{q} x_i = 1 \text{ with } 0 \leq x_i \leq 1 \text{ and i} = 1, 2, ..., \text{q} \tag{2.4}$$

Where q is the number of components (or factors) of a mixture.

2.2 Applications of the RSM in food industry

Nowadays, the response surface methodology (RSM) application in the optimization of different fields of food industry processes is common, due to its advantages compared with conventional methods. In this section, two applications have been illustrated.

2.2.1 Optimization of extraction processes

In recent years, numerous studies have been published in the literature about extraction of oil, protein, phenolic compounds, pigments, polysaccharides, hydrocolloids, etc. by applying the response surface methodology (RSM) in order to optimize the process conditions (Yolmeh and Jafari, 2017). There are several techniques for extraction of various bioactive compounds such as maceration, ultrasonic-assisted extraction (UAE), and microwave-assisted extraction (MAE) (Dorneles and Noreña, 2020; González et al., 2020; Matini et al., 2020). In these techniques, several factors including time, pH, temperature, reagents concentration, irradiation time, flow rate and solvent to solid ratio affect extraction yield and process efficiency which can be considered as independent variables in the response surface methodology (RSM). Maximizing the extraction yield has been the main target for these studies. Quality of analyte (target compound) is also important in addition to the yield, which the RSM can introduce in the optimal conditions for multiple-response processes. Randomization of experiments and replication on central point are important criteria that should be used in the designs to validate the RSM modeling. The validation of the predicted optimum conditions is another important issue when applying the RSM approach (Dean et al., 2017).

2.2.2 Application of the RSM in formulation processes

Since several factors are considered in the formulation of food products, researchers are seeking for optimization of each response (factor), which could be performed by the response surface methodology (RSM), as this approach is useful for multi-response processes (Yolmeh and Jafari, 2017). The response surface methodology (RSM) has been used for optimizing formulations of different foods such as functional wheat dextrin yogurt (Peerkhan and Nair, 2020), malted barley based symbiotic beverage (MISHRA et al., 2020), oat-based cookie dough (Mohd Basri et al., 2020), gluten-free cookies (Olawoye et al., 2020), gluten-free bakery products (Nieto-Mazzocco et al., 2020), gelatin-chitosan-pectin films (Yeddes et al., 2020), beef products for older consumers (Botinestean et al., 2020), cinnamon essential oil nanoemulsion (Pongsumpun et al., 2020) and kahweol and cafestol from roasted coffee (Bianchin et al., 2020).

Part II

Experimental Part

Chapter 3

Morphological, physicochemical and phytochemical characterization of Degla-Beida dates and evaluation of its antioxidant activity *in vitro*

In the present work, all the experiments were performed in the laboratory of Biomathematics, Biophysics, Biochemistry and Scientometrics (BBBS) of Bejaia's University, as well as in the research and development laboratory of Cevital company and the laboratory of quality control and compliance (QualiLab) of Bejaia. It was also the product of foreign collaborations with the department of chemical engineering, University of Seville in Spain, the department of drug sciences, University of Pavia in Italy, and the department of nutrition and food science, University Complutense of Madrid in Spain.

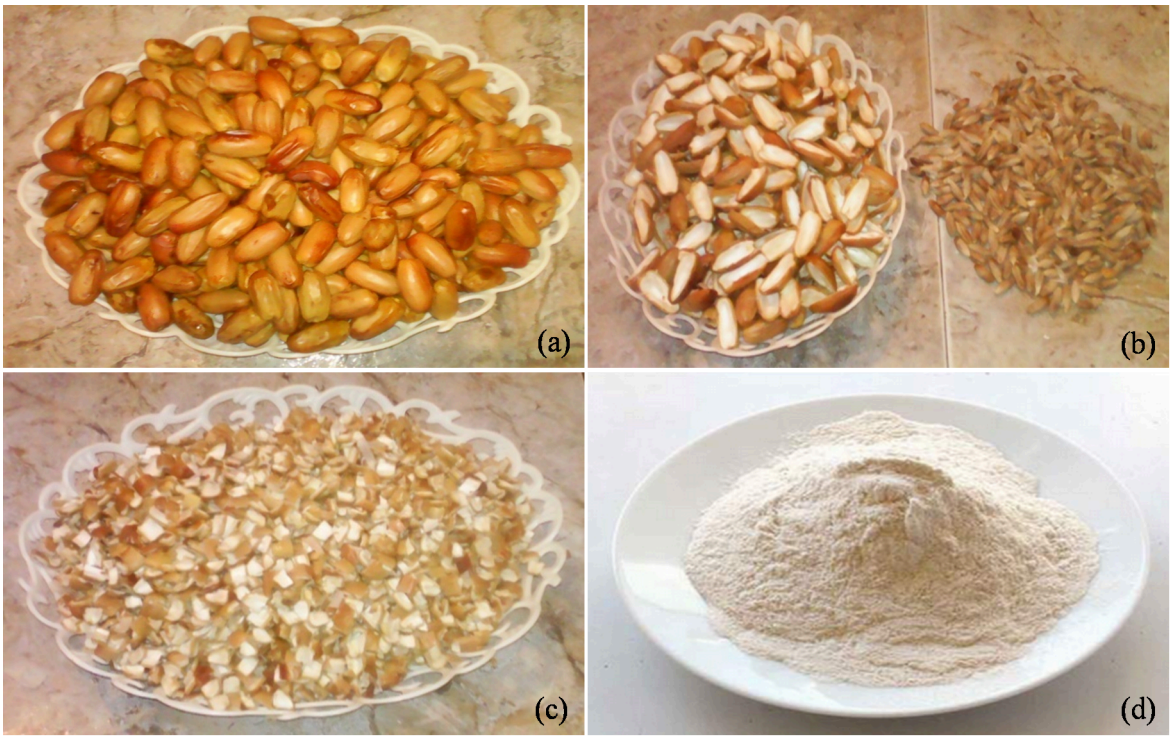
Date fruits, represent an inexhaustible source of bioactive compounds such as polyphenols. In this context, the objectives of this chapter were, to assess the morphological and physicochemical characteristics of the studied common date variety "Degla-Beida". Then, to determine the phytocomposition, by high performance liquid chromatography RP-HPLC-PDA-ESI-MS/MS, of the optimized phenolic extract by microwave-assisted extraction (MAE) using the response surface methodology (RSM), with comparison to the extracts obtained by ultrasound-assisted extraction (UAE) and conventional extraction (CE) methods. Finally, to test its antioxidant activity *in vitro*.

3.1 Material and methods

3.1.1 Chemicals and plant material

The chemicals used in this work were obtained from Sigma-Aldrich (represented by the Algerian chemical society, Setif, Algeria). The HPLC-grade water was obtained from LC-Pak^{TM} Millex system (Millipore Corporation, Billerica, MA, USA). Formic acid, citric acid, galacturonic acid, malic acid, gallic acid, MS grade methanol, glucose, sucrose and caffeine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Quinic acid, catechin, procyanidin B_1, procyanidin C_1, kaempferol glucoside, kaempferol, rutin, and quercetin rhamnoside were from PhytoLab (Vestenbergsgreuth, Germany).

Degla-Beida is the dry date variety used in this study, it is very common in the palm groves of south-eastern of Algeria and more precisely in Biskra (Photography 3.1). The choice of this variety is justified by its relative abundance on the national territory, its low market value, its long shelf life due to its dry nature, its taste and nutritional quality (source of energy), particularly represented by its richness in sugars.



Photography 3.1. Representation of the processing stages of "Degla-Beida" dates: washing and sorting (a), pitting (b), cutting and drying (c), grinding and sieving (d).

The dates were washed with tap water then with distilled water (Photography 3.1a), pitted with hands (Photography 3.1b), cuted into small pieces and dried in an oven at 40 °C until obtaining a constant weight (Photography 3.1c), then grounded in an electric grinder to obtain a fine powder (Photography 3.1d). The powder was passed through a standard sieves and stored in bottles in dark until use.

3.1.2 Morphological characterization

The morphological characterization of the date fruits was carried out as described by Djaoudene et al. (2019) and summarized below:

Date fruits

- The shape of the dates;
- The color at tamar stage;
- The consistency of the dates;
- The average weight of the dates;
- The average size of the dates.

Date seeds

- The shape of the seeds;
- The color of the seeds;
- The average weight of the seeds;
- The average size of the seeds.

N.B.

- The color was appreciated visually;
- The consistency by touch;
- The dimensions were determined using a calliper;
- The weight (whole pulp as well as the seeds) was determined using an analytical balance (±0.0001).

3.1.3 Physicochemical analyses

3.1.3.1 Moisture content and dry matter

The thermal drying method was used to determine the moisture content of the date fruit as described by Boulekbache-Makhlouf et al. (2013). 10 g of sample were placed in an oven at 105 °C until the stabilization of the weight. The moisture content was calculated by expressing the weight loss during drying as a fraction of the initial weight.

$$\text{Moisture content (\%)} = (W_0 \ / \ W_i) \times 100 \tag{3.1}$$

Where W_0 corresponds to the weight loss on drying (g) and W_i corresponds to the initial weight of the sample (g).

The determination of the dry matter was carried out by difference between the initial weight (100%) and the moisture content, the result was expressed as follows:

$$\text{Dry matter (\%)} = 100\% \text{ - \% moisture} \tag{3.2}$$

3.1.3.2 Ash content

The ash content was measured by incinerating two grams of date powder, in a calibrated crucible, in the muffle furnace at 550 °C for 4 h as described by Asma et al. (2020). The desiccator-cooled crucibles were weighed and the result was expressed as follows:

$$\text{Ash content (\%)} = [(P_2 - P_0) - (P_0 \ / \ P_1)] \times 100 \tag{3.3}$$

Where P_0: mass of the crucible empty, P_1: mass of the crucible empty + sample, P_2: mass of ash crucibles + ashes.

3.1.3.3 Color determination

The CIE laboratory coordinates (L*, a*, b*) were read directly into a glass cuvette with a Mini Scan XETM Spectrophotocolorimeter (HunterLab Inc., Reston, VA, USA). In this coordinate system, the L* value is a measure of brightness, ranging from 0 (black) to 100 (white), the a* value ranges from -100 (greening) to +100 (redness) and the b* value ranges from -100 (blue) to +100 (yellowing) (Turki et al., 2020).

3.1.3.4 Titratable acidity and pH

The acidity titration was performed automatically by an acidimeter and expressed as a percentage (%). For the pH, 10 g of sample were weighed and slurried in 75 mL of distilled water in a beaker. After calibrating the pH meter with pH 4, 7 and 9 buffer solutions, the electrodes were then immersed deeply into the prepared solution. The pH value was linked on the screen (Zarbakhsh and Rastegar, 2019).

3.1.3.5 Total sugar content (TSC) and soluble solid content (SSC)

According to the method described by Hanson and Phillips (1981), 400 μL of the extract (diluted in distilled water) was placed in the test tube, 2 mL of anthrone reagent was added and the solution was mixed. After incubation at 100 °C for 10 min, the absorbance was measured at 625 nm against a blank (made as reported for the sample but with 400 μL of distilled water), by using an UV-vis Spectrophotometer (SpectroScan 50, UK). Glucose was used as a standard for the calibration curve to express the total sugar content (TSC) as mg GE/mL (Annex 1).

The soluble solid content (SSC) was determined by measuring the refractive index of the date juice with a hand refractometer. The result was expressed as a percentage of soluble solids (Brix, %) in the juice at 25 °C (Zarbakhsh and Rastegar, 2019).

3.1.3.6 Quality index (r)

In 1961, Munier defined an index "r" of quality or hardness. It is equal to the ratio of sugar content on water content of dates. The calculation of this index makes it possible to estimate the degree of stability of the fruit and leads to the following classification: soft dates $r<2$, semi-soft dates $2<r<3.5$ and dry dates $r>3.5$.

3.1.4 Phytochemical analyses

3.1.4.1 Extraction of date polyphenols

Three extraction methods were used for the extraction of date polyphenols: microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and conventional extraction (CE).

3.1.4.1.1 Optimization of microwave-assisted extraction (MAE)

The microwave-assisted extraction (MAE) of date polyphenols were executed using a laboratory microwave system (MAXMOS23S, Maxipower, China). This apparatus operates at 2450 MHz with a maximum delivered power of 900 W and cavity dimensions of 28.1 cm (H) × 48.3 cm (L) × 38.7 cm (D). These experiments were conducted with date powder (1 g) and solvent added (water, ethanol, methanol and acetone) at different concentrations (20, 40, 60, 80 and 100% v/v). The samples were treated under different selected conditions (100, 300, 500, 700 and 900 W for the microwave power, 1/2, 1, 2, 3, 4 and 5 min for the irradiation time and 1:20, 1:40, 1:60, 1:80 and 1:100 g/mL for the solid-to-solvent ratio). Indeed, the effect of the type of solvent, the ethanol concentration (%), the microwave power (W), the irradiation time (min) and the solid-to-solvent ratio (g/mL) on the total phenolic extraction were studied. After each experiment, the extract was filtered through a filter paper and the volume of the solution was adjusted with the extraction solvent and stored at 4 °C. The parameters that had the greatest influence on the extraction of polyphenols using the microwave-assisted extraction (MAE) were selected for the response surface methodology study.

3.1.4.1.2 Ultrasound-assisted extraction (UAE)

The ultrasound-assisted extraction of date polyphenols was performed in an ultrasonic bath (mod.2510E-DTH, Mexico, USA), with working frequency fixed at 42 kHz, as described by Abu-Reidah et al. (2017) with slight modifications. 1 g of date powder was extracted with 20 mL of ethanol 50% (v/v), for 60 min at room temperature. Afterwards, the extract was filtered and the volume of the solution was adjusted with ethanol 50% and stored at 4 °C.

3.1.4.1.3 Conventional extraction (CE)

The dates' phenolic compounds were extracted according to the method of Al-Turki et al. (2010), with slight modifications. One gram of date powder was extracted with 20 mL of ethanol 50% (v/v). The mixed ethanol-sample solution was kept under magnetic stirring for 60 min. The extracts were then paper filtered, the final volume of the solution was adjusted with ethanol 50% and stored at 4 °C.

3.1.4.2 Total phenolic content (TPC)

The amount of total phenolics was determined as described by Georgé et al. (2005), using the Folin-Ciocalteu reagent and gallic acid as a standard. 500 μL of each extract were added to 2.5 mL of Folin-Ciocalteu (diluted 10 times). After 5 min, 2 mL of sodium carbonate (75 g/L) were added. The mixture was incubated at 50 °C for 15 minutes and the absorbance was measured at 760 nm. The results were expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW) (Annex 1).

3.1.4.3 Experimental design

The response surface methodology (RSM) was employed to explore the variables that affect the microwave-assisted extraction (MAE), this approach enables the overall number of experiments and possible interactions between the variables to be considered. A Box-Behnken design, three levels/three factors, with three replicates at the center point was developed to investigate the effect of three independent variables (X_1-Ethanol concentration, X_2-Microwave power and X_3-Iradiation time) on the extraction yield of the total phenolic compounds (TPC) from dates' sample. The independent variables were coded at three levels (-1, 0 and 1) and each level was selected based on the polyphenols stability under the extraction conditions. The complete experimental design consisted of 15 experimental points. The technique described above was used to obtain the surface response by fitting the data to a polynomial model and the effect of each factor while the interactions between the studied factors being also evaluated.

3.1.4.4 RP-HPLC-PDA-ESI-MS/MS analysis

The chromatographic analysis of the optimized phenolic extract, obtained by microwave-assisted extraction under optimal conditions (X_1-Ethanol concentration, X_2-microwave

power and X_3-irradiation time) suggested by the Design-Expert® software, were performed using a Thermo Finnigan Surveyor Plus HPLC apparatus equipped with a quaternary pump, a Surveyor UV-Vis PDA detector, and a LCQ Advantage max ion trap mass spectrometer (all from Thermo Fisher Scientific, Waltham, MA), coupled through an ESI source. The RP-HPLC-PAD-ESI-MS/MS data were acquired under positive and negative ionization modes, using Xcalibur software. With this aim, the ion trap operated in full scan (100-2000 m/z), data dependent scan and MS^n modes; in fact, when greater discrimination was required, additional targeted MS^2 and MS^n experiments were performed on the selected pseudomolecular ions.

To optimize the MS operating conditions a preliminary experiment was performed: 10 μg/mL caffeine (H_2O/MeOH: 50/50 with 0.1% formic acid) and 10 μg/mL gallic acid (H_2O/MeOH: 50/50 with 0.1% formic acid) solutions were directly infused in the ESI interface at a flow rate of 25 μL/min into the mass spectrometer as described in our article (Djaoud et al., 2020b). The optimized conditions were as follows: 60 arbitrary units of sheat gas, a capillary temperature of 220 °C, auxiliary gas 20 and 25 spray voltage 4.5 and 5 kV, capillary voltage -26.13 V and 35 V, respectively for negative and positive ionization mode.

The separation was achieved on a Kinetex XB RP18 80A (150 × 4.6 mm; 5 μm) column, protected by the corresponding guard column, both from Phenomenex, California, USA, operating at 25 °C. A gradient elution was executed with acidified water (0.1% formic acid) as the A mobile phase and methanol as the B mobile phase, at a flow rate of 0.3 mL/min. The elution gradient for the separation of the date polyphenols consisted of moving from: 10 to 70% B in 104 min, 70 to 80% B in 5 min, 80 to 100% B in 5 min, a 5 min isocratic of 100% B, 100 to 10% B in 5 min and finally an isocratic of 10% B for 6 min. The sample tray temperature was set at 4 °C and the injection volume was 5 μL. The chromatograms were recorded at λ 254, 280, 330 nm and the spectral data were collected in the range of 200-800 nm for all the peaks. The compounds were characterized on the basis of their UV-Vis and mass spectra, checking the molecular ion and the fragment ions against the fragmentation patterns of the standard molecules where possible and the molecules described in the literature.

3.1.5 Evaluation of the antioxidant activity

Several methods have been developed to assess the radical scavenging and the total antioxidant activities of phenolic extracts. In this chapter, four complementary methods were evaluated *in vitro* for the optimized extract by microwave-assisted extraction: the DPPH radical scavenging activity, the ABTS radical scavenging activity, the reducing power and the phosphomolybdenum assay.

3.1.5.1 DPPH radical scavenging activity

The ability of the extract to scavenge the free DPPH radicals was determined by the method of Milardović et al. (2006). 100 μL of the sample were mixed with 2.9 mL of the DPPH solution in methanol (6.10^{-5} M). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 515 nm.

The percentage of the activity was calculated according to the following equation:

$$\text{DPPH } (\%) = [(A_{cont} - A_{ext}) \ / \ A_{cont}] \times 100 \tag{3.4}$$

Where A_{cont} is the absorbance of the control (without extract) after 30 min and A_{ext} is the absorbance of the extract.

3.1.5.2 ABTS radical scavenging activity

The ABTS radical scavenging activity of the date phenolic extract was measured using the method given by Re et al. (1999). The ABTS radical cations ($ABTS^+$) were formed by reacting an aqueous solution of ABTS (7 mM) with an aqueous solution of potassium persulfate (2.45 mM). The mixture was left in the dark at room temperature for 12-16 h before use and then diluted with distilled water to obtain an absorbance of 0.700±0.005 at 734 nm. 30 μL of the sample were added to 3 mL of the ABTS radical solution at room temperature for 6 minutes and the absorbance was immediately recorded at 734 nm. The percentage of the activity was calculated according to the following equation:

$$\text{ABTS (\%)} = [(A_{cont} - A_{ext}) / A_{cont}] \times 100 \tag{3.5}$$

Where A_{cont} is the absorbance of the control (without extract) after 6 min and A_{ext} is the absorbance of the extract.

3.1.5.3 Reducing power

The reducing power was determined according to the method of Oyaizu (1986). The extract (1 mL) was mixed with 2.5 mL of 200 mmol/L of sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes. After adding 2.5 mL of 10% (w/v) trichloroacetic acid, the mixture was centrifuged at 650 rpm for 10 minutes.

The upper layer (5 mL) was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride, the absorbance was measured at 700 nm. The result were expressed in milligram of ascorbic acid equivalent per milliliter of extract (mg AAE/g DW).

3.1.5.4 Phosphomolybdenum assay

The antioxidant activity of the phenolic extract was also evaluated by the method of phosphomolybdate complex formation according to Prieto et al. (1999). This technique is based on the reduction of molybdenum Mo (VI) to molybdenum Mo (V) in the presence of antioxidant compounds and therefore the formation of a green phosphate/Mo (V) complex (Thangaraj, 2018). 0.3 mL of the extract was mixed with 3 mL of a solution containing 28 mM sodium phosphate (NaH_2PO_4), 0.6 M sulfuric acid (H_2SO_4) and 4 mM ammonium molybdate $(NH_4)2MoO_4$. The same process was repeated for the blank. The tubes were incubated in a water bath at 95 °C for 90 minutes. After the tubes had cooled to room temperature, the absorbance was measured at 695 nm using an UV-visible spectrophotometer. The antioxidant activity was expressed in milligram ascorbic acid equivalent per gram of dry weight (mg AAE/g DW).

3.1.6 Validation of the optimized solution and predicted model

To verify the suitability of the quadratic equation in predicting the optimal response value, the verification of the experiments were carried out under optimal conditions and compared to the predicted value. The extractions were made in triplicate.

3.1.7 Statistical analysis

The data were presented as mean values ± standard deviation (± SD) of three repeated experiments. The influence of each factor, on the total phenolic content (TPC) in the single factor experiment for the microwave-assisted extraction (MAE), was statistically assessed by the analysis of variance (ANOVA) and the Tukey's post hoc test, with a 95% confidence level using the XLSTAT Release 10 (Addinsoft, Paris, France).

3.2 Results and discussion

3.2.1 Morphological characterization

The morphological characteristics of the studied date cultivar *viz.*, fruit and seed shape, color, consistency, weight, length, width and their ratios, were represented in Table 3.1. Date fruit plasticity and texture were also determined and the results were expressed in grams (g) for the weight and in centimeters (cm) for the length and width.

The morphometric analysis results suggest that "Degla-Beida" variety is characterized by an ovoid shape, a beige color, a dry consistency, a low plasticity (hard) and a floury texture. Their seeds were also ovoid, with beige color and hard consistency. As described by Sayah (2010), the hard appearance of date fruits may be related to the date maturation stage, since dry dates do not pass through the stage routab. The morphological characteristics affect the physical properties and the organoleptic quality of date derived products as flour, vinegar, juice, ... etc.

Table 3.1. Morphological characterization of Degla-Beida dates.

Characteristic	**Value**
Date fruit (D)	
The shape	Ovoid
The color	Beige
The consistency	Dry
The plasticity	Hard
The texture	Floury
The weight (g)	6.85±0.76
The length (cm)	3.79±0.28
The width (cm)	1.75±0.11
Date pulp (P)	
The weight (g)	5.59±0.69
Date seed (S)	
The shape	Ovoid
The color	Beige
The consistency	Hard
The weight (g)	1.25±0.14
The length (cm)	2.44±0.16
The width (cm)	0.81±0.06
Ratios	
The weight (P/D)	81.61±0.10
The weight (S/D)	18.25±0.07
The length (S/D)	64.38±0.33
The width (S/D)	46.29±0.16
The weight (P/S)	4.47±0.12

Data are mean values ± standard deviation.

Date fruit weight constitutes a quality criterion which makes it possible to differentiate between varieties. Table 3.1 shows the average weight of the studied date variety (6.85±0.76 g) and their various constituents: pulp (5.59±0.69 g) and seed (1.25±0.14 g), the pulp yield (81.61%) as well as the percentage which represents the weight of the seed relative to the total weight of the fruit (18.25%). The values obtained for the length of the whole fruit (3.79±0.28 cm) and its width (1.75±0.11 cm), the length of the seed (2.44±0.16 g) and its width (0.81±0.06 cm), the ratios of the length (64.38) and the width (46.29) of the seed compared to the pulp and seed weight ratio P/S (4.47) were agree with those reported by Amellal (2008).

By comparing with other works, we noted that this results were close to those obtained by Sayah (2010) for the weight of the date (6.69 g), the pulp (6.04 g) and the seed (1.26 g), the length of the date (3.94 cm) and that of the seeds (2.47 cm), but they were slightly greater than those reported by Boukhiar (2009) for the average weight (6.19 g), the length (3.65 cm) and the width (1.78 cm) of the whole date, for the weight of the pulp (4.97 g) and the weight (1.22 g), the length (2.32 cm) and the width (0.80 cm) of the seed. According to Amellal (2008), a date is said to be of acceptable physical quality when the weight of the whole fruit is greater than or equal to 6 g, the weight of the date pulp is greater than or equal to 5 g, the length is greater than or equal to 3.5 cm and the diameter is greater than or equal to 1.5 cm. According to these criteria, the studied cultivar "Degla-Beida" dates have an acceptable physical qualities.

A recent study of Bedjaoui and Benbouza (2020) aimed at phenotypic diversity assessment among 26 Algerian date palm cultivars grown in Ziban region. As reported, there were significant differences between different morphological characteristics of the cultivars namely: the fruit weight (4.17-13.69 g), the fruit length (2.60-4.83 cm), the seed weight (0.51-2.82 g), the seed width (0.43-1.00), the seed length (1.60-2.97), the seed/fruit length ratio (0.52-0.73) and seed/fruit weight ratio (0.08-0.68). Actually, in groves, the fruit is the most used organ to identify and to name cultivars owing to the high morphological variation perceptible by the farmers. Among these fruit descriptors the size of dates. According to the results of the same authors, "Sbaa-Laroussa" cultivar had, the longest fruit, where "Sbaa" means "finger". This cultivar is grouped with Ain-el-Fas, Arechti and Feraounia cultivars whose fruits were well known by the farmers for being among the biggest dates in the Ziban region. However, qualitative parameters, like pinnae and date color, could also be used to distinguish between date cultivars.
Also, the study brought out the richness of Algerian date palm cultivars. However, this biodiversity is seriously threatened by diverse factors mainly the commercial one. Furthermore, during the prospections almost of the other cultivars' accessions were old and/or neglected so enable to produce offshoots and for many cultivars there are only few trees, that still available in the groves. The setup of *in situ* collections is highly recommended to preserve this biodiversity, as well as a well-planned strategy to promote other cultivars, in the region, for commercialization than Deglet-Noor.

3.2.2 Physicochemical analyses

The physicochemical characteristics of the studied date cultivar were as follow:

Table 3.2. Physicochemical analysis of Degla-Beida dates.

Analysis	**Value**
Moisture content (%)	12.93±0.10
Dry matter content (%)	87.14±0.10
Ash content (%)	1.67±0.67
pH	5.75±0.01
Acidity (%)	1.41±0.01
Total sugar content (%)	76.56±2.43
Solid soluble content (%)	21.73±0.17
Quality index	5.93±0.10
Color	
L*	88.93
a*	4.29
b*	18.15

Data are mean values ± standard deviation.

The moisture content of mature dates would depend on certain factors, the most important of which would be the frequency and the volume of irrigation, in side, the relative humidity at the harvesting time and at the storage site, in other sides. With respect of the moisture content of the studied cultivar, the value of 12.93±0.12% was obtained (Table 3.2). This value was relatively close to that found by Rambabu et al. (2020), for Maktoomi variety from United Arab Emirates and with that found by Mimouni and Oumelkheir (2015) for the same variety Degla-Beida (11.85%). The value recorded seems lower compared to that reported by Asma et al. (2020) for Hmira (25.42%) and Feggous (26.91%) varieties from Bechar Oasis, South-West of Algeria. Djaoudene et al. (2019), cited lower value than previously mentioned (11.34%) for Ourous variety from Ghardaia Southern of Algeria. The amount of moisture plays a role as a guide to the preservation method to inhibit the food spoilage. Moisture is also involved in determining the quality of the fruits. According to Agboola and Adejumo (2013), dry dates with low moisture content might not be acceptable to some consumers, whereas high moisture content may accelerate the deterioration of the date fruits. Dates that demonstrate low moisture levels were classified as semi-soft dates.

Furthermore, dates with moisture levels lower than 10% and higher than 20% are categorized as dry and soft dates, respectively (El-Sohaimy and Hafez, 2010). The dry matter of the dates used in this study was equal to 87.12±0.14% (Table 3.2). This value was similar to those indicated by: Benjeloul et Berragda (2014) and Mimouni (2015) for the same variety (88.18%). The high dry matter content of Degla-Beida variety can be explained by the low water content of the date itself, depending on the irrigation volume (Mimouni, 2015).

The ash content, with a percentage of 1.67±0.67%, was observed in the studied cultivar. This result seems to be relatively close compared to the data reported in previous studies. Asma et al. (2020), stated that date fruit contains ash of about 2%. The ash content depends, among other things, on the state of soil fertility and the amendments made. This result was higher than those reported by Djaoudene et al. (2019) for the three date cultivars from M'zab oasis, "Tazarzeit", "Ouaouchet" and "Tamezwert n'telet", which displayed ash contents of 1.25, 1.47 and 1.55%, respectively. Nevertheless, the ash content was lower than the values determined by Kchaou et al. (2013) for six-second grade cultivars of Tunisian dates that ranged from 2.11 to 3.06%.

The pH consists on measuring the acidity, the acids resulting from immature dates or the fermentation phenomenon increases dates' acidity and thus decreases the pH value (Asma et al., 2020). Raw date powder had a values of pH 5.75±0.01 and acidity 1.41±0.01, this pH was close to those reported by Lemine et al. (2014), which vary between 5-6. Comparable results were recorded by Ganbi and Hassan (2012), showed that the date pulp at complete ripening stage has a pH of 5.65. Di Cagno et al. (2017) repored a pH and acidity values of 6.22 and 0.2, respectively. The results registered by Asma et al. (2020) for Hmira variety (6.01) were lower than those found by Gourchala et al. (2015) for the same variety (5.46) and higher than our results. The acidity is proportional to the water content and therefore inversely proportional to the degree of maturity. The acidity of the cultivar under study was 1.41±0.01%, the values reported by Asma et al. (2020) were slightly higher. However, those obtained by Djaoudene et al. (2019) were lower, between 0.16 and 0.33%.

Many authors worked on several date varieties, claim that date sugars vary according to the variety, pollen, ripeness and climate. The sugar content of the studied date variety, expressed as a percentage of dry matter, was 76.56±2.43% DW. This value is between the maximum and the minimum of those found by: Abdul-Hamid et al. (2020) for nine date varieties from Madinah al-Munawarrah located at the North-Western part of Saudi Arabia (59.6-76.8% DW), Abdulrahman et al. (2020) for 21 date varieties from Land Races in Nigeria (64.55-82.85% DW), Awan et al. (2018) for Aseel, Dhaki and Zahidi Pakistani date cultivars (75.03, 77.42 and 80.91% DW, respectively), Ramchoun et al. (2017) for eight Moroccan date varieties (66.03–83.05% DW) and Lemine et al. (2014) for 28 cultivars from Adrar in Algeria (44.67-84.00% DW). Whilst, it was higher than those obtained by Turki et al. (2020), that varied between 27.8% DW for yellow fruits to 56.7% DW for the red variety from Nigeria. In general, the results reported by different authors depend in part on the method of analysis used. Nevertheless, everyone agrees that the total sugar content of dates is in the order of 60 to 80% of the pulp.

Total soluble solids content (TSS) gives the sucrose concentration of an aqueous solution (mainly constituted by sugars, with smaller amounts of organic acids, free amino acids, proteins, vitamins, essential oils and glucosides) with the same refractive index as the analyzed product (date). The important content reflects the richness of the date fruit in carbohydrate matters. The soluble solids content of the cultivar under study was 11.73±0.17 °Brix. This value was well above the maximums and below the minimums found in the 58 cultivars studied by Acourene and Tama (2001). This may indicate an average richness of the Degla-Beida dates in carbohydrate matter.

The CIELAB color parameters were shown in Table 3.2: L*=88.93, a*=4.29 and b*=1. Degla-Beida cultivar was characterized with a high L* value, this value was higher than that obtained by Seerangurayar et al. (2018) for Naghal date cultivar from Oman at tamar stage (L*=50.81, a*=0.47, b*=-2.52). According to Turki et al. (2020), the brightness values (L*) of the two studied date varieties varied between 35.02 for the red and 47.71 for the yellow fruits, whereas the a* parameter (redness) varied from 13.87 to 24.88. These significant differences between the varieties were probably due to

the presence of carotenoids. Djouab et al. (2016) determined the color parameters of Algerian red date specimens and found a values of 31.80 for L*, 37.80 for a* and 10.20 for b*. Amorós et al. (2014) determined the color parameters for Spanish yellow dates and the obtained values (L*=65.15, a*=22.50 and b*=47.80) were in accordance with the results found in our study. The quality index (total sugars/water content ratio), r=5.92±0.11, indicates that Degla-Beida is considered dry, index greater than 3.5.

3.2.3 Phytochemical analyses

3.2.3.1 Total phenolic content (TPC)

3.2.3.1.1 Effect of independent variables

The microwave-assisted extraction (MAE) is strongly affected by several parameters, such as the composition and the volume of the extractant, the microwave power, the irradiation time and the solid-to-solvent ratio.

Table 3.3. Preliminary study results of the microwave-assisted extraction.

Type of solvent		**Solvent concentration**		**Microwave power**		**Solid-to-solvent ratio**		**Irradiation time**	
Type	TPC (mg/g)	%	TPC (mg/g)	W	TPC (mg/g)	g/mL	TPC (mg/g)	min	TPC (mg/g)
EtOH	2.75±0.04^a	20	1.20±0.05^c	100	1.92±0.14^d	1:20	3.55±0.10^a	1/2	1.17±0.03^d
MeOH	2.12±0.07^b	40	1.93 ±0.07^b	300	2.36±0.07^c	1:40	3.48±0.08^a	1	3.03±0.04^b
Acetone	2.13±0.07^b	60	2.75±0.04^a	500	2.75±0.04^b	1:60	3.25±0.08^a	2	3.26±0.01^a
Water	1.37±0.03^c	80	1.87±0.05^b	700	3.38±0.05^a	1:80	2.82±0.14^b	3	2.68±0.03^c
		100	1.10±0.06^c	900	3.52±0.06^a	1:100	2.66±0.11^b	4	1.10±0.04^d
								5	1.00±0.15^d

Data are mean values ± standard deviation.

Values with different letters (a-b-c-d) are significantly different (Tukey, $p<0.05$).

Effect of extraction solvent

Ethanol and water are the most appropriate solvents used in food and nutraceutical industry, because they are easily removed from the final product and they increase the efficiency of phenolic acids and flavonoids' extraction (Moraes et al., 2013). Ethanol, methanol, acetone (at 60% v/v) and distilled water were used to investigate the effect of the extraction solvent on the yield of date polyphenols, as shown in Table 3.3. The highest polyphenols content was detected in ethanol extract (2.75±0.04 mg GAE/g DW) followed by methanol and acetone extracts then by distilled water extract.

The obtained results led us to conclude that ethanol was significantly ($p<0.05$) the best extraction solvent in term of TPC yield, so that consequently it was selected for the following experiments and for the response surface methodology (RSM) trial.

Effect of ethanol's concentration

The different aqueous ethanol mixtures have a different dielectric properties in the MAE, and this has a profound effect on the extraction of phenolic compounds (Setyaningsih et al., 2015). The effect of ethanol's concentration on the extraction efficiency was determined and the extraction yields were compared (Table 3.3). The highest extraction yield was obtained by using ethanol 60%, this concentration was selected for the following experiments and the range 40-80% was selected for the RSM study.

Effect of microwave power

Microwaves are a form of non-ionizing electromagnetic radiation with frequencies between 300 and 300 000 MHz. They are made up of two perpendicular oscillating fields, one magnetic and the other electric (Xiaokang et al., 2020). The oscillation fields cause molecular movement (ion conduction), migration of ions and dipole rotations. Ion conduction is the electrophoretic migration of ions such as salts under an alternating electric field. The resistance provided by the solution to the migration of ions generates friction which ultimately leads to heating of the solution (Tran et al., 2020).

Thus, to examine the effect of microwave power on the extraction yield of total phenolic compounds, various experiments were carried out. The results indicated a linear rise of the TPC yield from 100 to 700 W. This increase is because of a microwave power increase in that will improve the solubility of the sample for a better extraction efficiency. The microwave power's increment increased the dipole reactions which caused the heat generation in the mixture as a result of the power degeneration. After 700 watts, there was no increase in the yield. Consequently, 700 W was selected for the following experiments and the range 500-900 W was selected for the RSM trial.

Effect of irradiation time

The irradiation time is one of the key parameters. To study its effect, experiments were performed for different irradiation times (from 1/2 to 5 min). The results revealed that the TPC yield increased with the irradiation time increment up to 2 minutes. Beyond 2 minutes, a decrease in the yield was observed. The changes may be due to the spilling of the material during the pumping associated with the longer irradiation time and the possibility of polyphenol's denaturation. The time of two minutes was selected for the following experiments and the range 1-3 min was selected for the RSM study.

Effect of solid-to-solvent ratio

The sample to solvent ratio is another important parameter which when applied correctly could decrease the mass transfer barrier and subsequently enhance the extraction yield. It can be optimized using a single factor experiment. This is because the change in the solvent's volume due to different ratio, which affect the absorption of the microwave energy in the microwave-assisted extraction (MAE), making the basis of comparison inaccurate (Chan et al., 2014). The increase of the solid-to-solvent ratio, at constant solvent concentration (ethanol 60%), from 1:20 to 1:100 g/mL decreased the extraction yield of date polyphenols. To avoid solvent wastage, the optimum solid-to-solvent ratio for microwave-assisted extraction was selected and set to be 1:20 g/mL.

3.2.3.1.2 Optimization by RSM

To study the optimal conditions, a Box-Behnken experimental design was developed for the most dependent variables, which were: X_1-ethanol concentration used as an extractant, X_2-microwave power and X_3-irradiation time used to develop the extraction. A 15 factorial experimental design was used to study the significance of each variable and the correlation/interaction between them (Table 3.4).

Table 3.4. Box-Behnken design with observed responses of total phenolic content using microwave-assisted extraction.

Run	X_1	X_2	X_3	Recovery of TPC (mg GAE/g DW)	
				Experimental	Predicted
1	60	500	120	3.69±0.01	3.62
2	60	500	120	3.56±0.03	3.62
3	60	700	180	4.00±0.06	3.98
4	80	300	120	2.75±0.03	2.73
5	80	500	180	3.12±0.02	3.19
6	80	700	120	3.24±0.04	3.20
7	40	700	120	3.93±0.08	3.95
8	40	300	120	3.40±0.03	3.44
9	40	500	60	3.45±0.05	3.39
10	60	300	60	2.93±0.05	2.96
11	60	500	120	3.61±0.02	3.63
12	40	500	180	3.77±0.08	3.78
13	60	300	180	3.73±0.06	3.68
14	60	700	60	3.59±0.01	3.64
15	80	500	60	2.51±0.05	2.51

Data are mean values ± standard deviation.

The combined effect of ethanol concentration and microwave power on the TPC yield from date fruit was shown in the surface plot of Figure 3.1a. The polyphenols extraction rate proved to be very sensitive in regards to the solvent concentration modification. It was observed that the extraction yield increased by increasing the ethanol's concentration from 40 to 60% as well as by increasing the microwave power from 300 to 700 W. Nonetheless, a decrease tendency was observed by further increasing of ethanol's concentration from 60 to 80%. From the contour plot the maximal yield was predicted at 60% ethanol concentration and 700 W microwave power. Figure 3.1b. depicted the surface plot of the effect of two variables, namely ethanol's concentration and irradiation time on the total phenolic yield. The ethanol concentration demonstrated a parabolic effect on the response which increased from 40 to 60% and decreased from 60 to 80%. It was observed also that the extraction yield increased with the increase of the irradiation time as well as the ethanol's concentration. From this contour plot, the highest values of total phenolic content were obtained with ethanol 60% and 2 min of irradiation.

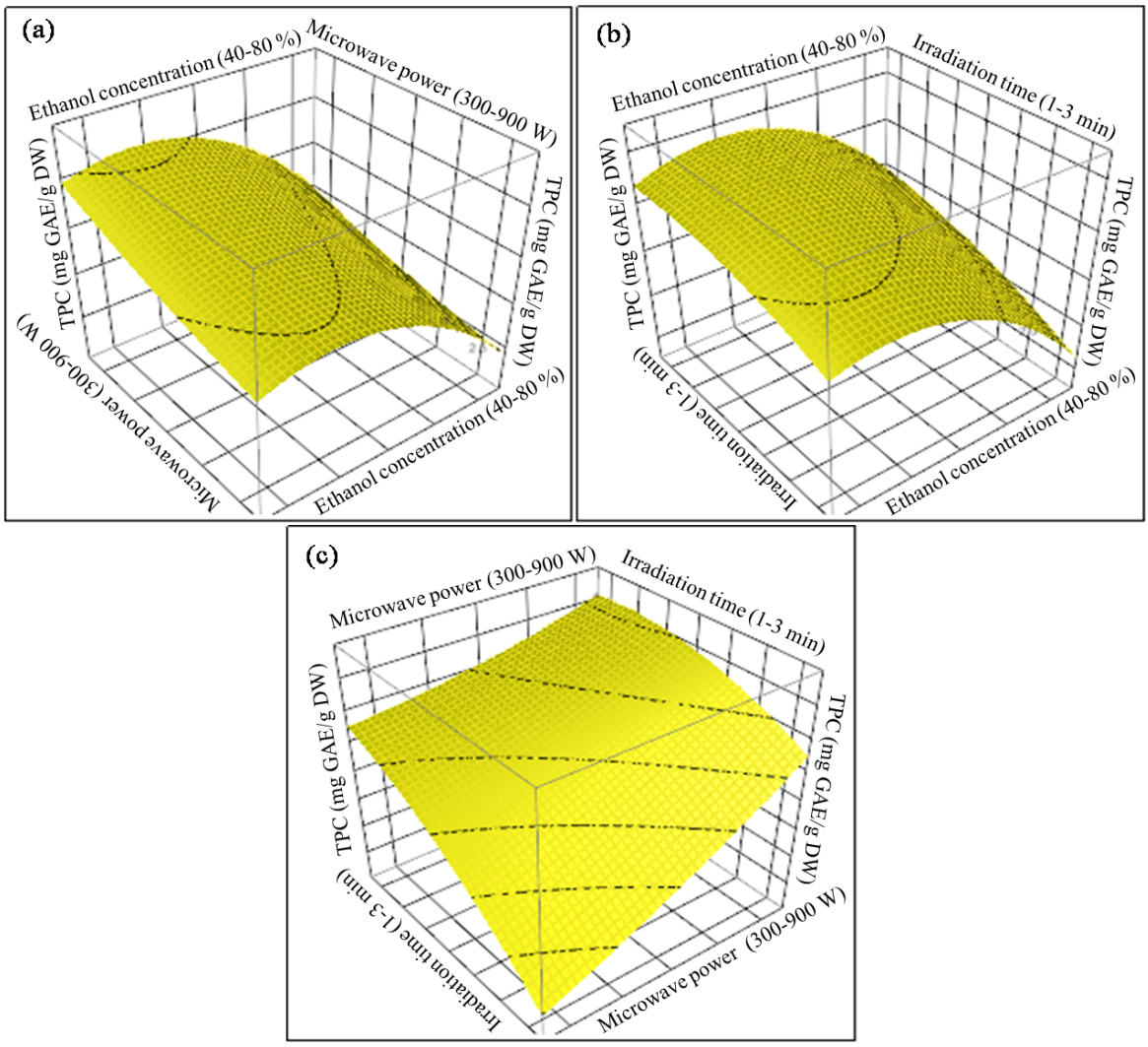


Figure 3.1. Response surface analysis for the TPC yield obtained by microwave–assisted extraction with respect to ethanol concentration and microwave power (a), ethanol concentration and irradiation time (b), microwave power and irradiation time (c).

The effect of microwave power and different levels of irradiation time on the TPC extraction can be predicted from the surface plot of Figure 3.1c. It was confirmed from this Figure that both of the independent variables had a strong effect on the extraction yield. The contour plot and the table 3.4 showed that the maximum extraction efficiency (4.00±0.06 mg GAE/g DW) was achieved under 700 W microwave power and 2 min irradiation time. Increasing the microwave power from 300 to 700 W and the irradiation time from 1 to 2 min significantly increased the TPC extraction rate from *Phoenix dactylifera* L. fruit. However, the further increase of irradiation time from 2 to 3 min caused a decrease of the extraction yield.

3.2.3.2 Model fitting and statistical analysis

The response surface methodology (RSM) is an empirical modeling technique, it calculates the correlation between actual and forecast outcomes. To attain a suitable model so as to optimize the microwave-assisted extraction, to understand the effect of the independent factors (X_1-ethanol concentration, X_2-microwave power and X_3-irradiation time) on date fruit TPC yield (Y) and to determine the optimum solutions, a Box-Behnken design (BBD) was used (Table 3.4). Table 3.4 presents the experimental data and the predicted values. The total phrnolic content (TPC) ranged from 2.51±0.05 to 4.00±0.06 mg GAE/g DW. The multiple regression analysis was applied to the experimental data, response variable and test variables were related to the following second-order polynomial equation according to the coded values:

$$Y(TPC) = 3.62 - 0.37X_1 + 0.24X_2 + 0.27X_3 - 0.01X_1X_2 + 0.07X_1X_3 - 0.10X_2X_3 - 0.32{X_1}^2 + 0.03{X_2}^2 - 0.09{X_3}^2 \tag{3.6}$$

Where Y is the yield of TPC calculated by the regression model and X_1, X_2 and X_3 were the coded variables.

To avoid poor or disingenuous response surface results, the adequacy of the model was checked using the regression analysis and the analysis of variance ANOVA (Sangamithra et al., 2015). ANOVA subdivides the total variation of the results into smaller parts coupled with the sources of variation to test the hypotheses about the independent factors (Swamy et al., 2014). Further, the Fisher's statistical test (F–test) analyzed the significance of each individual independent factor. The ANOVA's results (Table 3.5) for the quadratic model showed that the polynomial model was statistically significant, as suggested by the high F-value (F=50.80) and the low p-value (p<0.0001). The p and F values are used to check the significance of each coefficient, while they also indicate the interaction strength between independent variables. Higher F-value and lower p-value indicate that the corresponding coefficient is significant.

Table 3.5. Analysis of variance (ANOVA) for the experimental results obtained using microwave-assisted extraction.

	Sum of squares	***F*–value**	***p*–value**
Model	3.62	50.80	< 0.0001
X_1–Ethanol concentration	-0.37	13.79	< 0.0001
X_2–Microwave power	0.24	9.17	0.0003
X_3–Irradiation time	0.27	10.00	0.0002
X_1X_2	-0.01	-0.26	0.8062
X_1X_3	0.07	1.92	0.1134
X_2X_3	-0.10	-2.56	0.0506
${X_1}^2$	-0.32	-8.23	0.0004
${X_2}^2$	0.03	0.74	0.4918
${X_3}^2$	-0.09	-2.23	0.0763
Residual	0.03		
Lack of Fit	0.02	1.50	0.42
Pure Error	0.00		
R^2	0.99		
R^2_{adj}	0.97		
RMSE	0.07		

The R^2 and the adjusted R^2 confirm the adequacy and suitability of the model. The high values of the determination coefficient (R^2=0.99) and the adjusted determination coefficient (R^2_{adj}=0.97) showed a high degree of correlation between the experimental and the predicted values. The pure error was very low, indicating a good reproducibility of the obtained data. The model also showed that the lack of fit was statistically insignificant (p>0.05), which further confirmed the validity of the model.

At the same time, the results also demonstrated that the linear coefficients (X_2 and X_3), the quadratic coefficient (${X_2}^2$) and the interaction coefficient ($X_1 \times X_3$) have a significant effect, with low p-values (p<0.05). The other coefficients (X_1, ${X_1}^2$, ${X_3}^2$, $X_1 \times X_2$ and $X_2 \times X_3$) were found to be insignificant (p>0.05). Moreover, the ethanol's concentration (X_1) was the most significant parameter that influenced negatively the polyphenols extraction, followed by extraction time (X_3) and microwave power (X_2).

3.2.3.3 Investigating the model adequacy

Diagnostic plots ensure the model's adequacy and exhibit the correlation between actual and predicted results. The data points were close to the straight line, signifying a close agreement between the two data. It also suggests that the model is capable to select suitable operating conditions to extract polyphenol compounds from dates. Further, the normality assumption was verified by plotting a normal probability plot of the residuals. The data were found to lie near the straight line, so that it was concluded that the data were normally distributed.

3.2.3.4 Comparison between the extraction techniques

Extraction is the first step for the recovery of bioactive compounds from samples. Different extraction methods can be employed namely conventional and non-conventional. Conventional extraction methods have the disadvantages of high solvent requirements, thus, a large amount of solvent waste generation, as well as high energy consumption. These disadvantages can be overcome by the use of non-conventional extraction methods, including microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE)(Xiaokang et al., 2020). Microwave-assisted extraction (MAE) is an innovative technique presenting a special interest. Microwaves consist of a non-ionizing electromagnetic energy that is applied directly to the raw material. They transmit energy which penetrates into the biologic matrix and interacts with the polar molecules, mostly water, generating heat, the heat expands and disrupts the vegetal cell, favoring the extraction of intracellular phytochemical compounds. MAE is a technique frequently used to extract thermolabile compounds (Xiaokang et al., 2020). The ultrasound-assisted extraction (UAE) technique consists in using mechanic vibrations caused by sound waves with frequencies higher than 20 kHz. Sound waves are intrinsically different from electromagnetic waves, because the latter can propagate through the vacuum, while sound waves need a physical medium to propagate. The mechanic vibrations cause expansion and compression cycles in the medium, creating bubbles which collapse and cause cavitation, instantly creating a high local pressure and intense local heating. These fast changes induce the disruption and thinning of the cell membranes, consequently increasing the mass transfer rate of organic substances from the solid matrix to the solvent (Chemat et al., 2020; Mushtaq et al., 2020).

The optimum treatment parameters, ethanol concentration (50%), microwave power (700 W) and irradiation time (2.42 min) were selected for maximizing the yield. An mount of 2.82±0.05 and 2.27±0.04 mg GAE/g DW of polyphenols were extracted when UAE and maceration (60 min) techniques were used, respectively. MAE, after 2.42 min of extraction, not only reduced the extraction time and the solvent's consumption but also gave a higher yield (4.27±0.09 mg GAE/g DW) (Table 3.6).

Table 3.6. Comparison between MAE, UAE and CE techniques.

Extraction technique	**TPC mg GAE/g DW**
Microwave-assisted extraction (MAE)	4.27±0.09
Ultrasound-assisted extraction (UAE)	2.82±0.05
Conventional extraction (CE)	2.27±0.04

Data are mean values ± standard deviation.

Several scientific investigations report the applicability of the MAE for obtaining natural antioxidants, without generating undesirable residues (da Rocha and Noreña, 2020; Garcia-Vaquero et al., 2020; Sarfarazi et al., 2020). In general, the antioxidant activity of the extracts obtained by MAE was higher than the activity of the extracts obtained by conventional techniques, because the microwave treatment reduce the deterioration of the antioxidants in the extract (Weremfo et al., 2020). The MAE process is short, this fact makes it an attractive technique, for thermolabile compounds, although long extraction times can result in the degradation and consequent antioxidant capacity loss (Carbone et al., 2020).

The comparison of the obtained polyphenol contents to those reported in the literature showed that they were similar to some studies and in contrast to others. Indeed, the study of Hamad et al. (2015) performed on the extracts of twelve cultivars of *Phoenix dactylifera* L. from Saudi Arabia showed that the contents ranged from 10.4 to 22.1 mg GAE/100 g DW. In addition, the study done by Abdeldjabbar et al. (2020) showed that the total polyphenols contents of Ali Ourached date variety grown in Oued Souf region (South-East of Algeria) ranged from 1.95 to 16.98 mg GAE/100 g DW for chloroform, ethyl acetate and butanol fractions, except the crude extract, whose total polyphenols content was 124.25 mg GAE/100 g DW. The study of Abdul-Hamid et al. (2020) showed that the polyphenol contents of the aqueous extracts of nine varieties from Madinah al-Munawarrah located at the North-Western part of

Saudi Arabia were between 3.24 and 3.34 mg GAE/g DW. Othmani et al. (2020) tested the extracts of Tunisien Deglet Noor variety from Degache oasis, and they found that their total phenolic content varied between 104.972 and 131.105 mg GAE/100 g DW. The extract obtained by microwave-assisted extraction was selected to determine the phytocomposition and to evaluate the antioxidant activity of the studied variety.

3.2.3.5 RP-HPLC-PAD-ESI-MS/MS analysis

The RP-HPLC-PAD-ESI-MS/MS screening of date extract obtained by microwave-assisted extraction (MAE), was illustrated in the HPLC-UV (254 nm) chromatogram (Figure 3.2), which exhibited numerous peaks numbered following the elution order.

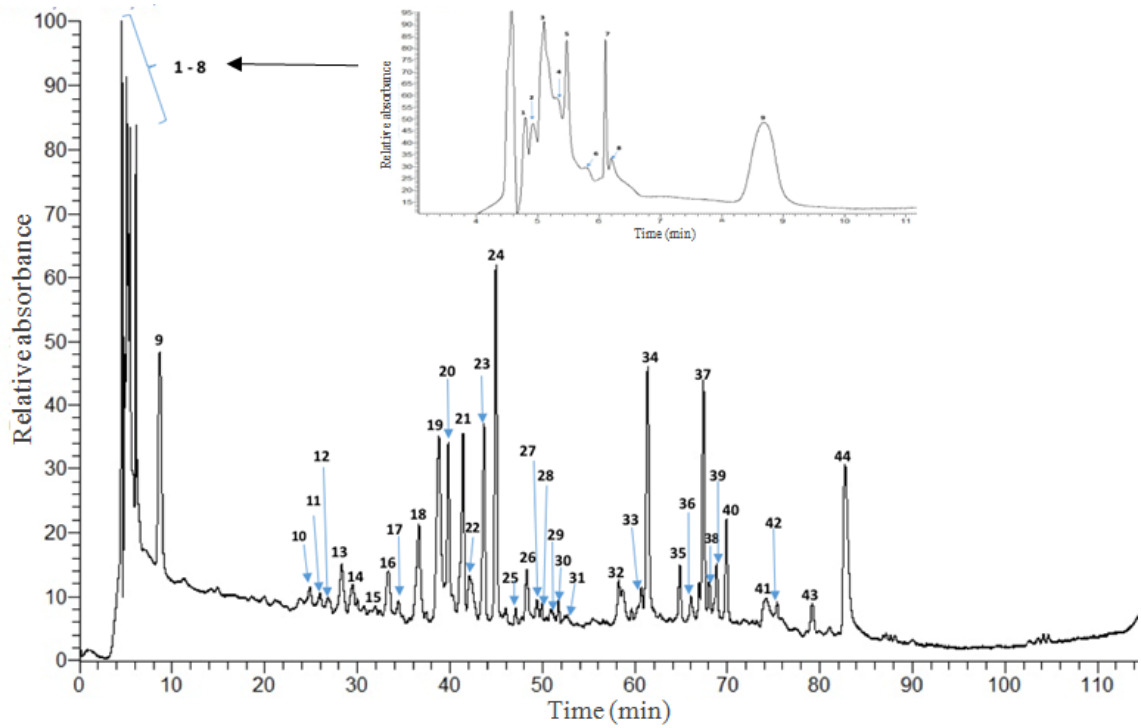


Figure 3.2. HPLC chromatogram of date extract, obtained by microwave-assisted extraction, recorded at 254 nm (inserted figure represents the magnification of the first 11 minutes of HPLC chromatogram).

Table 3.7 shows the different compounds (sugars, organic acids, nucleosides, carotenoids, proanthocyanidins, lignans, flavonols, flavones, saponin and sterol glucosides) that were grouped according to their structural similarity and following their elution order, as well as the Rt, MS data, UV_{max} and MS^n fragmentation pattern.

Table 3.7. RP-HPLC-PAD-ESI-MS/MS analysis of date extract obtained by MAE.

Peak No	(RT) (min)	λ Max (nm)	$[M-H]^-$	Fragments $[M-H]^-$ found	$[M+H]^+$	Fragments $[M+H]^+$ found	Proposed structure
SUGARS AND ORGANIC ACIDS							
1	4.80	226,265	387/341	341(100) → 179(<5)	/	/	Dihexoside
2	4.92	226,263	537	537 → 195(100), 177(10)	/	/	Gluconic acid disaccharide adduct
3	5.11	226,264	537	537 → 195(100), 177(10)	/	/	Gluconic acid disaccharide adduct
4	5.30	226,264	537	537 → 195(100), 177(10)	/	/	Gluconic acid disaccharide adduct
5	5.46	226,259	537	537 → 195(100), 177(10)	/	/	Gluconic acid disaccharide adduct
6	5.76	223,262	133	133 → 115(100), 113(10), 87(<5), 71(<5)	/	/	Malic acid
7	6.10	230,259	191	191 → 111(100), 173(30)	/	/	Citric acid
8	6.19	226,256	191	191 → 111(100), 173(30)	/	/	Citric acid
NUCLEOSIDS							
9	8.71	220,297	/	/	268	268 → 136(100), 106(<5)	Adenosine
CAROTENOIDS							
20	35.49	229,282, 322	/	/	541	541 → 379(100), 361 (40), 306(<5)	10-apo-B-carot-en-10-ol hexoside
21	36.66	202,232,326	/	/	571	571 → 409(100), 481 (30), 443(10), 391 (10)	Hexoside of 3-hydroxy-α-apo-10-carotenoic acid
23	38.34	233,286,326	/	/	703	703 → 541(100), 523 (20), 592(10) 541 → 379(100), 361 (40), 331(10), 460 (<5), 436(<5)	10-apo-β-caroten-10'ol dihexoside
24	39.07	232,281,324	/	/	733	733 → 571(100) 571 → 409(100), 481 (30), 443(10), 391(10)	Disaccharide of 3-hydroxy-α-apo-10-carotenoic acid
PROANTHOCYANIDINS							
13	26.20	228,277	/	/	579	427(100), 409(60), 291(50), 488(30)	Procyanidindimer
17	34	227,278, 305	/	/	867	577(100), 409(70), 427(60), 722(60), 715(40), 806(30), 699(30), 741(10), 623(<5), 553(30), 535(30), 451(30), 323(20)	Procynidintrimer
LIGNANS							
19	26.20	228,277	523	477(100), 361(15)	579	427(100), 409(60), 291(50), 488(30)	Secoisolariciresinol-O-hexoside
FLAVONOLS							
18	37.07	218,274, 305	449	287(100), 269(40), 431(30), 243 (10)	/	/	Dihydrokaempferol-hexoside
32	58.2	233,268, 333	/	/	595	449(100), 287(30)	kaempferol 3-O-rutinoside

(Table 3.7) contd ...

33	61.00	234, 268, 324	/	/	595	287(100), 433 (30)	kaempferol 3-O-rutinoside
34	61.31	255,352, 377	609	301(100), 343(15), 271(15)	611 179(<5)	515(100), 465(60), 303(60), 345(50), 417(40), 382(30), 494(30), 543(30)	Rutin
39	68.99	253,267,344	447	314(100), 357(20), 285(10), 179(<5), 152(<5), 399(<5), 329(<5)	/	/	Isorhamnetin-3-O-pentoside
40	69.94	255,268, 346,377	/	/	647	501(90), 331(100), 619(10), 489(10), 338(10)	Isorhamnetin-3-O-rutinoside
41	74.19	247,268, 338,377	543	301(100), 229(10)	/	/	Quercetin-hexosyl-sulfate
42	74.46	247,268, 338,377	543	301(100), 229(10)	/	/	Quercetin-hexosyl-sulfate
43	79	215,311, 376	519	314(100), 315(30), 357(15), 257(40), 491(20), 459(10), 223(10)	/	/	Isorhamnetinacetyl hexoside
FLAVONES							
23 35	38.34 64.20	233,286, 326 234,268, 332	/ /	/ /	595 609	577(100), 457(50), 475(20), 559(40), 463(100), 301(15)	6,8-C-diglucosylapigenin chrysoeriol-7-O-neohesperidoside
36	56.50	234,272, 319	/	/	463	301(100)	Chrysoeriolhexoside
37	67.45	234,268, 333	653	299(100), 284(50)	609	301(100), 463(30)	Chrysoeriol-7-O-neohesperidoside
44	82.74	251,347	541	299(100), 24(30)	543	301(100), 463(30), 504(10)	Chrysoeriolhexosyl-sulfate
						528(30)	
SAPONIN AND STEROL GLUCOSIDES							
25	47.02	202,238, 301,378	1111	949(100) 787(100) 741(80), 579(100), 353(30), 625(20) 579(100) → 417(100)	/	/	Eleutheroside E disacchardide formate adduct
26	48.29	202,240, 281,318, 377	787	741(100) → 579(100), 353(65) 579(100) → 417(100)	/	/	Eleutheroside E formate adduct
UNKNOWNS							
10, 11, 12, 15, 20, 22, 27, 28, 29, 30, 31	/	/	/	/	/	/	/
14	28	228,278, 309	/	/	449	335(100), 317(80), 274(30), 214(10), 143(<5)	/
16	34	224,278	/	/	/	317(100), 335(50), 336(20), 403(10), 463(10), 241(10), 189(<59)	/

3.2.3.5.1 Sugars and organic acids

The first compounds to elute from the C18 column, as it could be predicted, were the sugars and organic acids as they were detected only in the negative ionization mode (Table 3.7). Peak 1 was identified as a disaccharide with a m/z at 341, which breaks down to the 179[$MH^-162]^-$ fragment ions due to the loss of a hexosyl moiety (Sokeng et al., 2019). Peaks 2, 3, 4 and 5, instead all have the m/z of 537[$M^-H^-342]^-$ whose fragments lose a disaccharide to give m/z 195 which corresponds to gluconic acid. Peak 6 was identified as malic acid at a m/z 133, it breaks to its basic fragment at a m/z 115[$M^-H^-H20]^-$ due to the loss of a water molecule. Peaks 7 and 8 were identified as citric acid at a m/z of 191, whose fragments give different daughter ions at the m/z 111[$MH^-80]^-$ and 173[$MH^-H_2O]^-$ which are characteristic of its fragmentation pattern. The identification of sugars and organic acids was confirmed by comparison to the reference standards.

3.2.3.5.2 Nucleosids and carotenoids

Nucleoside and carotenoids were identified only in the positive ionization mode as described in Table 3.7. Peak 9 was identified as adenosine only in the positive ionization mode at m/z 268, this molecular ion breaks down to m/z 136[$M^+H^-132]^+$ due to the loss of a ribose moiety (Liu et al., 2008). Peak 20 at m/z was tentatively identified as 10-apo-β-caroten-10-ol hexoside having a molecular ion at m/z 541 which breaks to 10-apo-β-caroten-10-ol, its principal fragment at m/z 379[$M^-H^-162]^-$ due to the loss of a hexosyl moiety, 10-apo-β-caroten-10-ol then fragments at its turn to m/z 361[$M^+H^-H_2O]^+$ and 306[$M^+2H^-56]^+$ which are characteristic of this molecule (de Rosso and Mercadante, 2007). As previously seen, peak 21 at m/z 571 corresponds to 3-hydroxy-α-apo-10-carotenoic acid hexoside. Peak 23 instead at m/z 703 was tentatively identified as 10-apo-β-caroten-10-ol dihexoside, in fact, it suddenly breaks down to 10-apo-β-caroten-10-ol hexoside at m/z 541[$M^+H^-162]^+$ by the loss of a hexosyl moiety, the resulting 10-apo-β-caroten-10-ol hexoside then breaks down at its turn as previously described above. Peak 24 at m/z 733 was identified as a disaccharide of 3-hydroxy-α-apo-10-carotenoic acid, it fragments losing an hexosyl moiety to give its monosaccharidic form at m/z 571 which is 3-hydroxy-α-apo-10-carotenoic acid hexoside, then losses another hexose moiety giving rise to the aglicon-3-hydroxy-α-apo-10-carotenoic acid at m/z 409 which then fragments (Floss et al., 2008).

3.2.3.5.3 Proanthocyanidins and lignans

Like carotenoids, in this chemical characterization, proanthocyanidins were identified only in the positive ionization mode. Peak 13 at m/z 579 was identified as procyanidin dimer. However, peak 17 at m/z 867 was tentative identified as procyanidin trimer, it breaks down to its common fragments 577$[M+H]^-$. Peak 19 at m/z 523 corresponds to secoisolariciresinol-O-hexoside. These results were in accordance to the mass spectrum of the reference standard and to the study of Sokeng et al. (2019).

3.2.3.5.4 Flavonols and flavones

Regarding flavonols and flavones, they were identified in the positive as well as in the negative ionization modes. Peak 18 at m/z 449 was identified as dihydrokaempferol-hexoside in the negative ionization mode. As seen from Table 3.7, this molecular ion fragments to its basic fragment ions at m/z 287$[M^-H^-162]^-$ which is dihydrokaempferol due to the loss of the sugar moiety, 269$[M^-H^-180]^-$ due to the loss of the sugar and a water molecule, and 431$[M^-H^-H_2O]^-$ due to the loss of a water molecule (Mena et al., 2012). Peak 23 was identified in the positive ionization mode as 6,8-C-diglucosyl apigenin, it was fragmented as reported in Table 3.7. Peaks 32 and 33 were identified as kaempferol-3-O-rutinoside with the molecular ion at m/z 595 in the positive ionization which fragments to ions at m/z 449$[M^+H^-146]^+$ and 287$[M^+H^-308]^+$ due to the loss of rhamnosyl and rutinosyl respectively (Bamawa et al., 2016).

Peak 34 with molecular ions at the m/z 609 and 611, detected in negative and positive ionization modes respectively, was identified as rutin, due to its fragmentation pattern which includes fragment ions typical of quercetin derivatives. Due to the loss of the disaccharide rutinose (fragment ion at m/z 302), it corresponded to $[M^-H^-\alpha$-L-rhamnopyranosyl-(1→6)-β-D-gluco-pyranose$]^-$. Peaks 35 and 37 with the respective m/z of 607 and 609 in the negative and positive ionization modes, were identified as chrysoeriol-7-O-neohesperidoside, in both modes, it fragments losing the sugar moiety neohesperidoside (made of rhamnose and glucose) to form the fragment ion chrysoriol at m/z 299 and 301 in the negative and positive ionization modes respectively. Instead, peak 36 at m/z 463 was identified only in the positive ionization mode, it was tentatively identified as chrysoeriol hexoside, it fragments losing the sugar to form the fragment ion chrysoriol at m/z 301$[M^+H^-162]^+$.

A comparison to the reference standard (rutin) confirmed this result. Peak 39 at m/z 447 was identified only in the negative ionization mode as isorhamnetin-3-O-pentoside, it breaks down to its principal fragment at m/z 314[$M^-2H^-132]^-$ due to the loss of the sugar moiety (Schieber et al., 2002). Peak 40 at m/z 647 was identified in the positive ionization mode as isorhamnetin-3-O-rutinoside, it fragments generating the ions at m/z 501[$M^+H^+Na^-146]^+$ and 331[$M^+H^+Na^-316]^+$ by losing a rhamnosyl unit and isorhamnetinaglicone, respectively (de Bertoldi et al., 2012). Peaks 41 and 42 at m/z 543 were identified in the negative ionization mode as quercetin-hexosyl-sulphate, this molecular ion breaks to give its principal fragment at m/z 301[$M^-H^-242]^-$ due to the loss of an hexosyl-sulphate moiety. Peak 43 at m/z 519 was identified in the negative ionization mode as isorhamnetin acetyl-hexoside, it fragments to its basic ions 314[$M^-2H^-204]^-$, 315[$M^-H^-204]^-$ due to the loss of an acetyl-hexoside, it also gives the fragment at m/z 357[$M^-H^-162]^-$ due to the loss of a sugar moiety, others fragments typical of this molecule were reported in Table 3.7 (Sobeh et al., 2016). Peak 44 was identified as chrysoeriolhexosyl sulphate, it has an m/z of 541 in the negative ionization mode which fragments tochrysoeriol at m/z 299[M^-H^-hexosylsulphate]$^-$ while in the positive mode it has an m/z of 543 which principally fragments tochrysoeriol at m/z 301[M^+H^-hexosyl sulphate]$^+$ and chrysoeriol hexoside at m/z 463[$M^+H^-SO_4]^+$.

3.2.3.5.5 Saponin and sterol glucosides

From the RP-HPLC-PDA-ESI-MS/MS analysis, eleuthoroside E formate adduct and disaccharide formate adduct were identified (Table 3.7). Peak 25 at the m/z 1111 was identified as eleuthoroside E disaccharide formate adduct, it fragments losing the disaccharide and the formic acid to give the fragment ion eleuthoroside at the m/z 741 which then fragments as previously described (Xiao et al., 2018). In fact, peak 26 at the m/z 787 in the negative ionization mode breaks down releasing formic acid to give the fragment ion at the m/z 741 which corresponds to eleuthoroside E. Eleuthoroside E then breaks in turn to the give the fragment ions 579 and 417 due to the loss of one and two glucose moieties, respectively.

3.2.3.5.6 Unknowns compounds

Peaks 10, 11, 12, 14, 15, 16, 20, 22, 27, 28, 29, 30 and 31 were not identified either in the negative or in the positive ionization modes (Table 3.7).

Numerous studies were focused on the identification of phenolic compounds in dates' extracts by HPLC analysis. Indeed, the study of Alshwyeh (2020) showed the phenolic profiling of various Saudi Arabian native date palm (*Phoenix dactylifera*) cultivars (Ajwa, Khalas Alkharj, and Al-Qasim). Saleh et al. (2011) assessed the rutin, caffeic acid and catechin contents of the methanolic extracts of *Phoenix dactylifera* L. cultivars grown in Saudi Arabia. Dhaouadi et al. (2011) quantified the phenolic compounds, gallic acid, vanillic acid, cinnamic acid, 3,4-dicaffeoylquinic acid, 5-O-caffeoylshikimic, caffeoylsinapylquinic acid, caffeic acid, and coumaric acid, in the aqueous extract of Deglet-Nour variety grown in Tunisia. Similarly, the phenolic profile of hydro-acetonic extracts of three Tunisian date cultivars was established by Kchaou et al. (2016) where gallic acid, vanillic acid, caffeic acid, syringic acid, coumaric acid, ferulic acid, sinapic acid and rutin were identified. The analysis of phenolic compounds of four Omani date varieties by HPLC revealed the presence of gallic acid, coumaric acid, caffeic acid, vanillic acid and syringic acid (Al Harthi et al., 2015). The chromatographic separation of aqueous and ethanolic extracts from Egyptian date cultivars revealed the presence of gallic acid, tannic acid and ferulic acid, respectively. In contrast, the study reported the absence of cinnamic acid (El Sohaimy et al., 2015). The differences between the obtained results and those in the literature can be attributed to the geographical origin of the fruits (Al Harthi et al., 2015). The extraction method and solvent could also be at the origin of the variations in phenolic compounds of the date extracts (Amiour and Hambaba, 2016; Mrabet et al., 2016; Shahdadi et al., 2015).

3.2.4 Evaluation of the antioxidant activity *in vitro*

Oxidative stress, one of the main reasons behind different pathological conditions of human body, is originated from an imbalance between body's intrinsic defense and generation of free radicals. These unstable free radicals and reactive oxygen species (ROS) originate either from normal metabolic processes in human body or from external sources (X-rays, industrial chemicals, cigarette smoking, air pollutants etc.) and damage cellular macromolecules like protein, lipid and DNA through electron pairing resulting in a number of pathological conditions including degenerative diseases, cancer and inflammatory diseases (Zihad et al., 2021).

Antioxidant ability of natural products could occur through several mechanisms such as oxidizing enzymes inhibition, metal chelating, hydrogen and electron donation and the activation of singlet oxygen. So, several assays were necessary for a more complete evaluation of antioxidant activities of natural agents (Movahhedin et al., 2016). Polyphenols are compounds with one or more hydroxyl groups attached to the benzene ring. This structural feature provides a stronger acidic character to phenol than does to other alcohol groups. This chemical reactivity is responsible for the antioxidant character of polyphenols. The ability of polyphenols to capture free radicals is largely dependent on the number of hydroxyl groups (Tanase et al., 2019).

In order to evaluate date antioxidant properties of the optimized extract obtained by MAE, DPPH and ABTS radical scavenging activities, reducing power and phosphomolybdenum assay were determined. Both DPPH and ABTS are among the most popular methods used in quantifying antiradical activity. The principle of the analyses is based on the color change of the solution as radicals were quenched by antioxidant compounds which can be quantitatively measured from the changes in absorbance (Sadeer et al., 2020). As shown in Table 3.8, the antioxidant potential of the phenolic extract in term of DPPH and ABTS free radical inhibition was 58.72±0.29 and 50.77±0.64%, respectively. The comparison of these results with those reported in the literature shows that differences vary from one study to another. This shows the ability of date fruits to scavenge the different free radicals in different systems. They can be a useful therapeutic agents for the treatment of pathological damages related to free radicals (Hussain Mallhi et al., 2014).

The reducing properties are generally associated with the presence of reductones, which have the capacity to donate an electron to free radicals and convert them into more stable. Furthermore, reductones can react directly with peroxides and also with certain precursors and prevent the formation of peroxide (Zihad et al., 2021). The reducing power assay measures the reduction of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-azoniacyclopenta-1,4-diene chloride to blue ferrous complex by antioxidants (Haida and Hakiman, 2019).

The reducing capacity may serve as an indicator of the potential antioxidant activity of date fruits, the reducing power of the phenolic extract was 1.88±0.09 mg AAE/g DW (Table 3.8). Zineb et al. (2012) reported values ranged between 2.06 and 4.21 mg AAE/g DW of various Algerian date fruits.

The total antioxidant capacity assay can be used to determine the capacity of antioxidants through the formation of phosphomolybdenum complex (Haida and Hakiman, 2019). The optimized phenolic extract exhibited an antioxidant capacity of 23.14±0.14 mg AAE/g DW, as shown in Table 3.7. The results reported by Kchaou et al. (2013) for Tunisian date varieties, using water as extracting solvent, ranged from 17.49 (Bejo) to 39.94 mg AAE/100 g FW (Deglet Nour). According to Abbes et al. (2013), the antioxidant activity of three Tunisian date varieties Allig, Kentichi and Deglet Nour was of 118.75, 131.77 and 135.97 mg AAE/100 g FW, respectively.

Table 3.8. Total phenolic content and antioxidant activity of the optimized extract obtained by microwave-assisted extraction.

Test	**Value**
Total phenolic content (mg GAE/g DW)	4.27±0.09
DPPH (%)	58.72±0.29
ABTS (%)	50.77±0.64
Reducing power (mg AAE/g DW)	1.88±0.09
Phosphomolybdenum assay (mg AAE/g DW)	23.14±0.14

Data are mean values ± standard deviation

3.3 Conclusion

The use of common dates, with low market value, for the extraction of beneficial phytonutrients such as phenolic antioxidants, not only provides health benefits but also adds values to the waste. The aims of this chapter were to analyse the morphological and physicochimical properties of Degla-Beida dates, and to characterize the metabolic profile, by RP-HPLC-PAD-ESI-MS/MS, of the phenolic extract obtained by an optimization of microwave-assisted extraction (MAE) using the response surface methodology (RSM) that was the best method for extracting date phenolic compounds compared to ultrasound-assisted and conventional extractions (UAE and CE), and evaluating the *in vitro* antioxidant activity of the extract in question. The antioxidant activity of the optimized extract was evaluated and compared using the following methods: the DPPH and ABTS radical scavenging activities, reducing power and phosphomolybdenum assays. This study scientifically validated the use of dates in traditional medicine, and revealed their interest in the context of exploitation in the field of biotechnology. The future works should focus on the optimization and large scale extraction of the selected phytonutrients from dates, and their use as food additives.

Chapter 4

Conventional and green technologies for the preparation of date syrup using the response surface methodology

In Algeria, there are few date processing plans to valorize the high genetic diversity of the phoenicultural orchard. Important quantities of common dates (*Phoenix dactylifera* L.) with low comercial value are generated in each campaign, their chemical composition is similar to that of commercial dates. These dates, constitute a raw material for the development of many products with high added value as syrup, locally called "Rob or Dibs Tamr" which occupies a privileged place within the Saharan population. Currently the possibilities of biomass valorization by technological processes represent a solution of choice for the use of agricultural products of low commercial value.

This chapter aims to valorize the common date variety named Degla-Beida, which is often poorly exploited. In this context, we tried to prepare syrups from this secondary date variety and to evaluate the effect of alternative extractions (microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE)) and conventional extraction (CE) or water bath extraction (WBE) methods on its total sugar content (TSC), using the response surface methodology (RSM). Then, the analysis of its individual sugars was performed by high performance liquid chromatography (HPLC-RID). Date syrup is a nutritious and functional food with health benefits to consumers, the benefit becomes more economical when this food is made from by-products.

4.1 Material and methods

4.1.1 Extraction of date juice

Three methods were used for the extraction of date juices: microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and conventional extraction (CE) or water bath extraction (WBE).

4.1.1.1 Optimization of microwave-assisted extraction (MAE)

A microwave oven with a laboratory system (MAXMOS23S, Maxipower, China) working at 2450 MHz frequency with a cavity dimensions of 28.1 cm (H) × 48.3 cm (W) × 38.7 cm (D) was used. Ten grams (10 g) of date powder were mixed with 60, 70, 80, 90, 100, 120 and 140 mL of distilled water, respectively. The samples were treated under microwave irradiations in an intermittent way, *i.e.* irradiation-cooling-irradiation at power input of 100, 300, 500, 700 and 900 W with a fixed irradiation and cooling time of 1/2, 3/4, 1, 2, 3, 4 and 5 min, respectively. After irradiation, the extract was filtered under vacuum, centrifuged at 4000 rpm for 15 min (NF200, NÜVE, Turkey), then adjusted with distilled water. At the end, the extract was stored at 4 °C.

4.1.1.2 Optimization of ultrasound-assisted extraction (UAE)

Date powder (10 g) was immersed into 60, 70, 80, 90, 100, 120 and 140 mL of distilled water. The extraction process was performed using an ultrasonic bath (2510E-DTH, Mexico, USA), with working frequency fixed at 42 kHz and different ultrasonic temperatures: 30, 40, 50, 60 and 69 °C. After 15, 30, 45, 60 and 90 min, the extract was filtered under vacuum, centrifuged at 4000 rpm for 15 min (NF200, NÜVE, Turkey), then adjusted with distilled water. Finally, the extract was stored at 4 °C.

4.1.1.3 Optimization of conventional extraction (CE) or water bath extraction (WBE)

Conventional extraction in water bath was carried out as follows: 10 g of date powder were extracted for 1, 30, 60, 90, 120 and 180 min using a thermostatic water bath shaker (WNB22, Memmert, Germany), with 60, 70, 80, 90, 100, 120 and 140 mL of distilled water at different shaking speeds (30, 50, 70, 90 and 110 rpm, respectively)

and different temperatures (40, 50, 60, 70, 80 and 90 °C, respectively). The extract was filtered under vacuum, centrifuged at 4000 rpm for 15 min (NF200, NÜVE, Turkey), then adjusted with distilled water. The obtained extract was stored at 4 °C until use.

4.1.2 Total sugar content (TSC)

The most commonly used method for measuring carbohydrate concentrations is the anthone method, as described by Hanson and Phillips (1981) (Section 3.1.3.5).

4.1.3 Experimental design

The response surface methodology (RSM) was applied to determine the optimal processing conditions generated by the statistical software package JMP (10.0.0 version, SAS Institute, USA). Three extraction methods with different processing variables including: X_1-Microwave power, X_2-Irradiation time and X_3-Solid-to-liquid ratio for the MAE, X_1-Ultrasonic temperature, X_2-Ultrasonic treatment time and X_3-Solid-to-liquid ratio for the UAE, and X_1-Temperature, X_2-Treatment time, X_3-Solid-to-liquid ratio and X_4-Shaking speed for the CE, were chosen based on the results of the single factor experiments. Then, the effect of these key variables was investigated using the Box–Behnken experimental design (BBD), to develop a response surface quadratic model for describing the extraction process, then the yield of total sugar content (TSC) was taken as the response of the design experiments. According to this design, a total of 15 experiments were performed for the MAE and UAE, however, 27 experiments were performed for the CE containing three replicates at the center point. The regression analysis of the experimental data was performed to establish the empirical second order polynomial models as shown in the following equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i \, x_i + \sum_{i=1}^{k} \beta_{ii} \, x_i^2 + \sum_{1 \leq i \leq j}^{k} \beta_{ij} \, x_i \, x_j + \varepsilon \tag{4.1}$$

Where Y is the measured response, which is in our case the total sugar content (TSC), β_0 is a constant, β_i is the linear coefficient (main effect), β_{ii} is the quadratic coefficient, β_{ij} is the two factor interaction coefficient, x_i and x_j are the independent variables.

4.1.4 Preparation of date syrups

To prepare date syrups, the obtained juices from each extraction method were concentrated at 70 °C, using an oven, until having syrups at 80 °Brix.

4.1.5 Identification of individual sugars by HPLC-RID

A high performance liquid chromatography was used for the analysis of individual sugars (fructose, glucose and sucrose) from the optimized samples. The separation was carried out on a cationic (Ca^{++}) or cation exchange column, the mobile phase (the eluent) was the distilled water and the detector was a refractive index detector (RID). Sugars were extracted from different samples (0.1 g) by stirring for 10 min in a 100 mL flask with 20 mL of distilled water, and then adjusted to 1.5 mL. The samples were filtered through membrane filters (0.45 μm) and analyzed by liquid chromatography (LC). The results were expressed in percentages (%) by the software calculation of the area of each peak. The molecules of glucose, fructose and sucrose were characterized by their retention time (by comparison with their respective standards).

4.1.6 Statistical Analysis

Each extraction trial and all the analyses were carried out in triplicate, the data were reported as mean values ± standard deviation (SD). The influence of each factor on the total sugar yield in the single factor experiments for the microwave-assisted, ultrasound-assisted and conventional or water bath extraction methods was statistically assessed by ANOVA analysis of variance and Tukey's post hoc test, with 95% confidence level using the XLSTAT Release 10 (Addinsoft, Paris, France).

4.2 Results and discussion

Before carrying out the Box-Behnken design (BBD) approach, preliminary experiments were undertaken to select the lower, middle and higher levels (-1, 0 and +1) for each factor. The results of the preliminary experiments were shown in Table 4.1. Distilled water was selected as the extraction solvent for the response surface methodology trials.

Table 4.1. Results of single-factor experiments of MAE, UAE and WBE methods.

MAE							
Microwave power		**Irradiation time**		**Solid-to-liquid ratio**			
W	TSC (g/L)	min	TSC (g/L)	g/mL	TSC (g/L)		
100	104.17±5.28^{c}	1/2	110.99±3.20^{d}	1:6	73.39±9.02^{d}		
300	190.39±3.80^{b}	3/4	155.58±8.31^{b}	1:7	111.45±7.87^{c}		
500	220.25±1.49^{a}	1	220.63±1.85^{a}	1:8	117.14±5.29^{c}		
700	175.90±4.21^{b}	2	230.45±1.28^{a}	1:9	160.74±6.37^{b}		
900	118.58±4.44^{c}	3	205.75±5.18^{a}	1:10	182.01±8.57^{a}		
		4	156.01±7.87^{c}	1:12	184.64±6.27^{a}		
		5	132.42±4.58^{c}	1:14	185.89±7.34^{a}		
UAE							
Temperature		**Extraction time**		**Solid-to-liquid ratio**			
°C	TSC (g/L)	min	TSC (g/L)	g/mL	TSC (g/L)		
30	33.50±3.14^{c}	15	103.45±4.36^{c}	1:6	81.47±4.25^{c}		
40	92.73±7.34^{b}	30	131.89±2.31^{ab}	1:7	94.34±4.91^{bc}		
50	108.82±10.96^{b}	45	153.09±6.78^{bc}	1:8	120.83±5.36^{abc}		
60	13.51±10.35^{a}	60	196.82±6.90^{a}	1:9	154.27±6.62^{ab}		
69	13.81±10.65^{a}	75	165.51±5.34^{a}	1:10	190.46±9.88^{a}		
		90	119.37±7.55^{bc}	1:12	191.75±7.90^{a}		
				1:14	190.58±5.29^{a}		
CE							
Temperature		**Extraction time**		**Solid-to-liquid ratio**		**Shaking speed**	
°C	TSC (g/L)	min	TSC (g/L)	g/mL	TSC (g/L)	rpm	TSC (g/L)
40	144.08±5.58^{b}	1	151.46±3.21^{d}	1:6	152.56±4.32^{c}	30	156.54±10.12^{b}
50	168.47±8.21^{b}	30	217.98±8.14^{ab}	1:7	175.87±7.22^{bc}	50	162.96±10.34^{b}
60	180.81±8.34^{b}	60	229.87±3.98^{b}	1:8	182.07±5.74^{ab}	70	177.00±7.54^{ab}
70	189.64±4.98^{ab}	90	174.44±6.09^{bc}	1:9	205.32±3.47^{ab}	90	195.64±6.17^{ab}
80	202.26±5.39^{ab}	120	160.20±4.58^{cd}	1:10	216.85±4.17^{a}	110	229.82±3.77^{a}
90	217.83±8.74^{a}	180	155.45±6.00^{cd}	1:12	215.02±6.95^{a}		
				1:14			

Data are mean values ± standard deviation.

Values with different letters (a-b-c-d) are significantly different (Tukey, $p<0.05$).

4.2.1 Optimization of microwave-assisted extraction (MAE)

4.2.1.1 Effect of independent variables

Effect of microwave power Low microwave power reduces the extraction efficiency. However, too much power results in wasted energy. Therefore, an optimal microwave power should be determined. The total sugar content (TSC) significantly increased when the microwave power increased from 100 to 500 W, where as the other extraction parameters were constant (1 min for the irradiation time and 1:10 g/mL for the solid-to-liquid ratio). The total sugar recovery was parabolic with a maximum value at 500 W. However, as the microwave power continued to increase up to 900 W, the total sugar content decreased. Based on these results, the microwave power range 300-700 W was selected for the response surface methodology (RSM) trials, and 500 W was fixed for the next single factor experiments on the influence of the irradiation time.

Effect of irradiation time The extraction time is an important parameter in the extraction of phytochemicals. It has two phases: 1) the dissolution of soluble components on the surface of the sample particles, and 2) the mass transfer of the solute from the plant matrix into the solvent by diffusion and osmotic processes (Şahin and Şamlı, 2013). When the contact time between the solvent and the sample is longer, its absorption is improved, and the permeability of the cell wall is increased, which allows the solvent to penetrate the plant material in order to accelerate the release of the compounds of interest. However, a very long time can result a decrease in the efficiency of the extraction process. Therefore, the irradiation time must be appropriate. The extraction was carried out at different irradiation times, whereas other parameters were constant (500 W microwave power and 1:10 g/mL solid-to-liquid ratio). The TSC significantly increased as the irradiation time increased from 1/2 to 2 min. Then, the recovery was maximized at 2 min and declined moderately with further increases in irradiation time (5 min). Since shorter extraction time is also favorable to reduce energy costs, the range 1-3 min was selected for the RSM study, while 2 min of irradiation were used for the last single factor trials dealing with a varying solid-to-liquid ratio.

Effect of solid-to-liquid ratio For an efficient extraction, the solvent volume must be sufficient to ensure a complete immersion of the materials, and an extraction solvent deficiency can lead to lower extraction yields (incomplete extraction) of ingredients (Sun and Wang, 2008), although redundant solvent may also lead to lower extraction yields and solvent waste (Zou et al., 2011). Therefore, the solid-to-liquid ratio must be appropriate. The extraction was carried out at different solid-to-liquid ratios, whereas other extraction parameters were constant (500 W for the microwave power and 2 min for the irradiation time). The extraction yield of the total sugar content significantly increased as the solid-to-liquid ratio increased within the range of 1:6-1:10 g/mL. However, the extraction yield did not change significantly when the solid-to-liquid ratio continued to increase (1:14 g/mL). Based on the statistical analysis, the range 1:8-1:10 g/mL was selected for the response surface methodology (RSM) optimization.

4.2.1.2 Optimization by RSM

The experiments were carried out according to the conditions indicated in Table 4.2. The combined effect of microwave power, irradiation time and solid-to-liquid ratio on the total sugar content (TSC) were shown in Figure 4.1. The relationship between the response and the experimental variables can be illustrated graphically by plotting a three-dimensional response surface plots. The vertical axes show the yield of total sugars, and each of the two horizontal axes represents two of the three independent variables. In every plot, the factor not represented by the two horizontal axes was fixed at its 0 coded level. As can be seen in the Figure 4.1 and Table 4.2, all the studied factors have a significant influence on the TSC, *i.e.* a significant increase in the recovery of total sugars with the elapse of irradiation time when microwave power was increased. Figure 4.1a, illustrated the interaction effect of microwave power and irradiation time, when the solid-to-liquid ratio was set at its 0 level (1:9 g/mL). The increase in the microwave power and irradiation time increased the total sugar content up to a maximum of 224.57 g/L. However, a prolonged extraction time with microwave power gave a reduced yield, which was also noticed during our preliminary study.

Table 4.2. Box–Behnken design with the observed responses of total sugar yield.

	MAE					**UAE**				
Run	**X_1**	**X_2**	**X_3**	**TSC (g/L)**		**X_1**	**X_2**	**X_3**	**TSC (g/L)**	
				Experimental	**Predict**				**Experimental**	**Predict**
1	300	2	1:10	212.22±0.74	211.47	40	60	1:10	186.17±2.04	187.24
2	500	3	1:10	228.27±2.04	230.21	60	60	1:8	178.15±1.96	177.08
3	300	1	1:9	202.35±1.19	202.81	50	30	1:8	161.11±1.34	162.69
4	300	2	1:8	176.42±2.04	177.89	50	90	1:8	155.56±1.11	156.67
5	300	3	1:9	199.38±2.41	198.21	60	90	1:9	189.38±1.19	189.34
6	500	3	1:8	192.47±3.52	192.18	50	60	1:9	180.99±0.57	179.42
7	700	2	1:8	180.00±2.06	180.76	60	60	1:10	201.11±0.98	202.73
8	500	2	1:9	224.57±1.30	222.59	50	30	1:10	190.12±6.02	189.01
9	500	2	1:9	220.49±2.04	222.59	40	30	1:9	171.36±4.26	171.40
10	700	2	1:10	222.72±2.26	221.25	50	60	1:9	180.37±1.28	179.42
11	500	1	1:10	230.49±1.50	230.79	60	30	1:9	191.36±1.30	190.85
12	500	1	1:8	196.67±1.48	194.74	40	60	1:8	154.07±1.96	152.45
13	500	2	1:9	222.72±2.63	222.59	40	90	1:9	168.15±1.70	168.66
14	700	3	1:9	208.03±1.67	207.56	50	90	1:10	192.35±0.86	190.77
15	700	1	1:9	204.94±1.90	206.11	50	60	1:9	176.91±2.99	179.42
	CE									
Run	**X_1**	**X_2**	**X_3**	**X_4**	**TSC (g/L)**					
					Experimental	**Predict**				
1	75	60	70	1:10	219.14±0.77	216.34				
2	75	30	90	1:7	184.44±3.16	184.87				
3	90	60	90	1:10	228.64±0.93	230.38				
4	75	60	110	1:10	229.75±2.04	228.58				
5	75	90	110	1:8.5	194.44±1.70	194.35				
6	60	60	90	1:10	206.79±0.57	207.87				
7	75	60	90	1:8.5	202.22±1.34	201.52				
8	75	60	90	1:8.5	204.20±2.41	201.52				
9	75	30	70	1:8.5	185.56±1.11	186.90				
10	75	90	90	1:7	178.52±1.70	178.71				
11	75	30	90	1:10	213.46±2.63	214.15				
12	75	90	70	1:8.5	184.44±1.70	184.89				
13	75	30	110	1:8.5	198.15±3.90	198.96				
14	75	60	70	1:7	186.67±1.70	185.70				
15	60	60	110	1:8.5	185.93±1.11	187.19				
16	90	60	90	1:7	209.63±1.96	209.80				
17	60	60	90	1:7	164.69±0.86	164.21				
18	90	60	110	1:8.5	218.03±2.23	216.55				
19	90	30	90	1:8.5	209.26±2.89	208.66				
20	75	60	110	1:7	194.32±1.50	194.98				
21	75	90	90	1:10	213.21±1.67	213.67				
22	60	60	70	1:8.5	169.38±1.71	171.74				
23	90	90	90	1:8.5	200.74±0.37	201.28				
24	60	30	90	1:8.5	173.21±0.93	170.54				
25	90	60	70	1:8.5	210.86±1.67	210.48				
26	75	60	90	1:8.5	198.15±2.31	201.52				
27	60	90	90	1:8.5	172.84±1.07	171.30				

The effect of the interaction between microwave power and solid-to-liquid ratio on the TSC when the irradiation time was set at its 0 level (2 min) were presented in Figure 4.1b. The results indicated that the yield increased with the increase of microwave power and solid-to-liquid ratio at the beginning of the extraction. The recovery reached its maximum (224.57 g/L) at 500 W and 1:9 g/mL solid-to-liquid ratio during the MAE process. The increase in microwave power above 500 W led to a decrease in the extraction recovery.

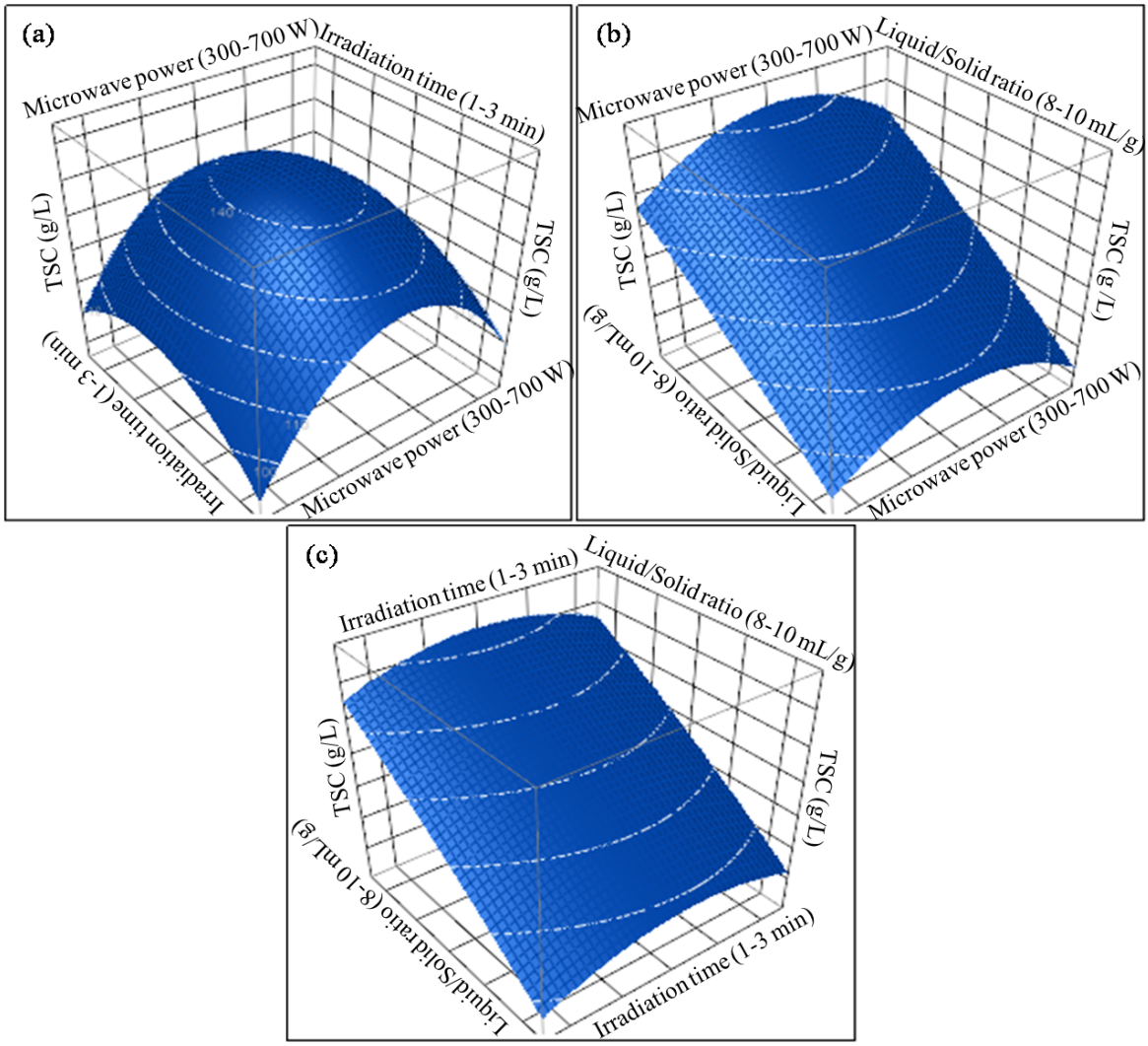


Figure 4.1. Response surface analysis for the TSC obtained by microwave-assisted extraction with regard to microwave power and irradiation time (a), microwave power and solid-to-liquid ratio (b), irradiation time and solid-to-liquid ratio (c).

The effect of the interaction between irradiation time and solid-to-liquid ratio at 500 W (0 level) on the extraction yield were presented in Figure 4.1c. The total sugar content increased roughly from 192.47 to 230.49 g/L when the ratio increased from 1:8 to 1:10 g/mL and the irradiation time varied for 1-2 min. Then, a decline was observed after 2 min. The predicted yield (233.25±3.59 g/L) obtained by the optimal conditions (microwave power 530 W, irradiation time 1.99 min and solid-to-liquid ratio 1:10 g/mL) was very close to the value predicted by the model (233.80±1.90 g/L).

4.2.2 Optimization of ultrasound-assisted extraction (UAE)

4.2.2.1 Effect of independent variables

The propagation of ultrasonic waves and cavitation effect are two key factors resulting in the enhancement of the efficiency of bioactive compounds extraction (Nguyen et al., 2021). The cavitational effect, which increases mass transfer and results in close interaction between the solvent and plant tissues, is the principle of ultrasound-assisted extraction. Briefly, the implosion of cavitation bubbles generates macro-turbulence, high-velocity inter-particle collisions and perturbation in micro-porous particles of the biomass, which accelerates the internal diffusion. Moreover, the collapse of cavitation bubbles in plant tissue surfaces produces micro-jets, leading to tissue disruption and good solvent penetration into the tissue matrix (Vilkhu et al., 2008). Usually, ultrasonic power is believed to be the driving force for the complete dispersion of solvent into the sample (Han et al., 2011). In this work, the ultrasonic power was fixed to 42 KHz.

Effect of temperature In the case of temperature, its increases can influence the membrane structure of the plant cells, making them less selective (Wang et al., 2018). Additionally, at high extraction time, sugars can be degraded. Different temperatures were investigated (30-69 °C) at a fixed ultrasonic treatment time (30 min) and solid-to-liquid ratio (1:10 g/mL). The TSC significantly increased when the ultrasonic temperature increased from 30 to 60 °C. However, the extraction yields did not change significantly when the ultrasonic temperature continued to increase (69 °C). Based on these observations, moderate ultrasonic temperatures of 40, 50 and 60 °C were selected as the lower, middle and higher levels, respectively, to apply in the RSM optimization.

Effect of extraction time When the ultrasonic treatment time was at a low level (15 min), the yield of total sugars increased to a certain value at 60 min with a fixed temperature (60 °C) and solid-to-liquid ratio (1:10 g/mL). After which, the yield decreased with increasing ultrasonic treatment time up to 90 min. The highest yield was obtained at 60 min. Based on these results, the 30-60 min range was used for further experimentation using ultrasound-assisted extraction (UAE) for the response surface methodology optimization, while 60 min was kept for the next single-factor trials.

Effect of solid-to-liquid ratio A positive effect on the total sugar yield was recorded when the solid-to-liquid ratio was 1:10 g/mL (Table 4.1). There was an increase in the yield with the increase of solid-to-liquid ratio from 1:6 to 1:10 g/mL. However, the total sugar content (TSC) was not significantly affected when the solid-to-liquid ratio continued to increase (1:14 g/mL). Based on the statistical analysis, the range 1:8-1:10 g/mL was finally selected for the response surface methodology (RSM) optimization.

4.2.2.2 Optimization by RSM

In this optimization, we have evaluated the effect of X_1-ultrasonic temperature, X_2-ultrasonic treatment time and X_3-solid-to-liquid ratio on the total sugar yield (Y). The obtained data from 15 runs of experiments using ultrasound-assisted extraction (UAE) were shown in Table 4.2. The three-dimensional response surface plots in Figure 4.2 illustrate the relationship between the extraction yield of total sugar and the experimental variables.

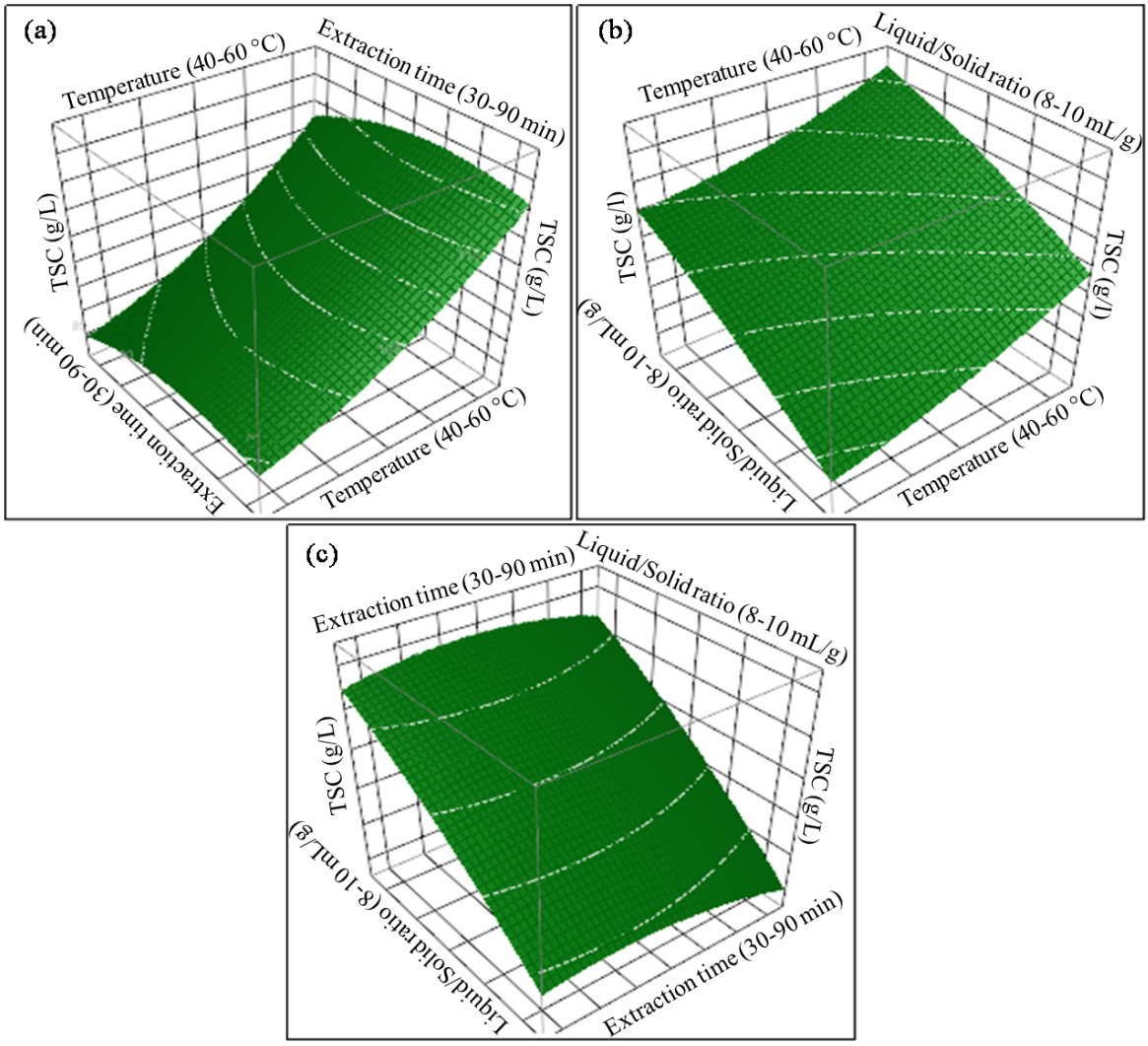


Figure 4.2. Response surface analysis for the TSC obtained by ultrasound-assisted extraction with regard to ultrasonic temperature and extraction time (a), ultrasonic temperature and solid-to-liquid ratio (b), extraction time and solid-to-liquid ratio (c).

The effect of ultrasonic temperature and ultrasonic treatment time on the extraction yield of date total sugars at a constant solid-to-liquid ratio (0 level), was showed in the Figure 4.2a. The extraction yield was gradually increased with temperature and ultrasonic treatment time from 171.36 to 191.36 g/L and peaked at 60 °C and 30 min. The extraction yield began to decrease beyond 60 min. The appropriate solid-to-liquid ratio (1:10 g/mL) had a positive effect on the extraction yield of total sugars, as showed in the response surface plots for the effect of solid-to-liquid ratio on the extraction yield (Figures 4.2b et 4.2c) at constant ultrasonic treatment time and constant ultrasonic temperature, respectively.

The extraction yield increased along with the increase in the solid-to-liquid ratio from 1:8 to 1:10 g/mL and ultrasonic temperature from 40 to 60 °C to reach 201.11 g/L (Figure 4.2b). It was also observed that a simultaneous increase in the solid-to-liquid ratio from 1:8 to 1:10 g/mL and ultrasonic treatment time of 30-60 min increased the total sugar content (TSC) from 178.15 to 201.11 g/L. After this increase, the yield began to decrease beyond 60 min (Figure 4.2c).

The predicted yield (202.89±5.80 g/L) obtained using the optimal conditions (ultrasonic temperature 60 °C, treatment time 68.10 min and solid-to-liquid ratio 1:10 g/mL) was very close to the value predicted by the model (202.04±3.40 g/L).

4.2.3 Optimization of conventional extraction (CE) or water bath extraction (WBE)

4.2.3.1 Effect of independent variables

Effect of temperature It is known that high temperature can facilitate the release of sugars into the headspace by overcoming energy barriers that bind them to the matrix. Different temperatures were investigated from 40 to 90 °C at a fixed treatment time (30 min), shaking speed (110 rpm) and solid-to-liquid ratio (1:10 g/mL). As indicated in this paragraph, most sugars were extracted efficiently at 90 °C. Based on these results, the temperature range 60-90 °C was selected for the RSM trials and 90 °C was fixed for the next single-factor experiments on the influence of extraction time.

Effect of extraction time The treatment time is one of the most important parameters in the water bath extraction method. In this study, different extraction times were investigated, from 1 to 180 min, at a fixed temperature (90 °C), shaking speed (110 rpm) and solid-to-liquid ratio (1:10 g/mL). An increase in the efficiency was observed when the extraction time increased from 1 to 60 min, and then it began to decrease beyond 60 min. Thus, the optimum equilibrium time was determined, and the range of 30-90 min was selected for the response surface methodology (RSM) trials, while 60 min were kept for the next single-factor trials.

Effect of solid-to-liquid ratio Different solid-to-liquid ratios from 1:6 to 1:14 g/mL were selected to be compared for their effectiveness in the date sugar extraction, at a fixed temperature (90 °C), extraction time (60 min) and shaking speed (110 rpm). The solid-to-liquid ratio of 1:10 g/mL presented the highest yield for the investigated compounds. Thus, based on these preliminary results, the solid-to-liquid ratio of 1:10 g/mL was selected for the extraction of total sugars in the subsequent experiments while the range 1:7-1:10 g/mL was selected for the response surface methodology trials.

Effect of shaking speed The addition of shaking speed as a fourth factor can increase the extraction yield. As a consequence, the extraction efficiency was improved due to the favored diffusion of analytes to the headspace. Different shaking speeds (30, 50, 70, 90 and 110 rpm) were used to investigate their influence on the conventional procedure at a fixed temperature (90 °C), extraction time (60 min) and solid-to-liquid ratio (1:10 g/mL). An increases in the total sugar yield were observed with the increasing of the shaking speed from 30 to 110 rpm. Thus, higher level resulted in higher extraction efficiency. Therefore, 110 rpm shaking speed was chosen as the best condition in this regard while the range 70 to 110 rpm was selected for the RSM trials.

4.2.3.2 Optimization by RSM

The experimental design and the corresponding response data for the total sugar content (TSC) from *Phoenix dactylifera* L. for the conventional extraction were presented in Table 4.2, with twenty seven experiments. The effect of X_1-temperature, X_2-treatment time, X_3-solid-to-liquid ratio and X_4-shaking speed on the extraction yield (Y) was investigated, and the results were shown in Figure 4.3. The three-dimensional response surface plots in this figure illustrate the relationship between the extraction yield and the experimental variables. The plots were generated by plotting the response using the z-axis against two independent variables (X_1 et X_2) while keeping the other two independent variables (X_3 et X_4) at their zero level. These results indicate that all tested parameters have a great impact on the extraction yield of date sugars.

The obtained profile for the effect of temperature and treatment time on the sugar yield, at a fixed shaking speed and solid-to-liquid ratio was shown in Figure 4.3a.

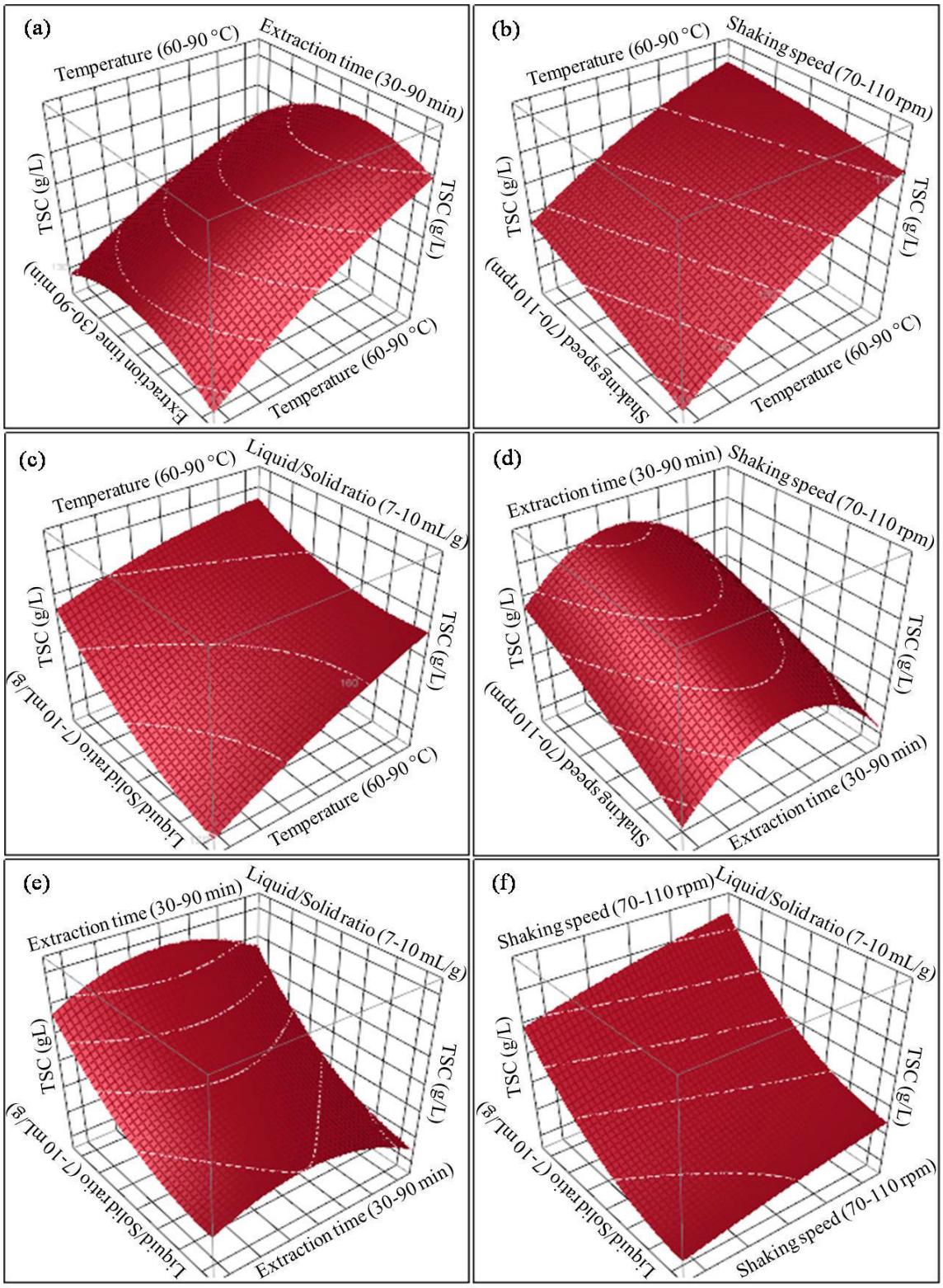


Figure 4.3. Response surface analysis for the TSC obtained by conventional extraction with regard to temperature and extraction time (a), temperature and shaking speed (b), temperature and solid-to-liquid ratio (c), shaking speed and solid-to-liquid ratio (d).

The extraction yield of date sugars gradually increased with temperature and treatment time, and peaked at approximately 90 °C and 30 min to reach 209.26 g/L. Further increase in treatment time enhanced the degradation of the investigated compounds, which resulted in the decrease of the total sugar content (TSC) to 200.74 g/L.

With respect to the interaction between the temperature and the shaking speed when the treatment time and the solid-to-liquid ratio were set at their 0 levels, were presented in Figure 4.2b. By increasing the temperature and the shaking speed from 60 to 90 °C and 70 to 110 rpm, respectively, the total sugar content (TSC) increased from 169.38 to 218.03 g/L, and peaked at approximately 90 °C and 110 rpm shaking speed. Figure 4.1d. illustrates the interaction effect of the extraction time and the shaking speed, when the temperature and the solid-to-liquid ratio were set at their 0 levels (75 °C and 1:8.5 g/mL). The increase in the extraction time and shaking speed increased the total sugar content (TSC) up to a maximum of 204.20 g/L. However, a prolonged extraction time reduced it, this result was also noticed during our preliminary study.

The response surface plots (Figures 4.3c-3e-3f) for the effect of solid-to-liquid ratio on the extraction yield of date sugars, at a constant temperature, treatment time and shaking speed, shows that an appropriate solid-to-liquid ratio (1:10 g/mL) had a positive effect on the extraction yield (Figure 4.3c), when the 3D response surface plot was developed for the recovery of the TSC with varying temperature and solid-to-liquid ratio. It can be observed that a maximum recovery of the TSC (228.64 g/L) was achieved with a temperature of 90 °C at a solid-to-liquid ratio of 1:10 g/mL. In the latter case, the extraction was increased when the treatment time was lower than 60 min and decreased when it exceeded 60 min. The interactive terms, in the model, were not significant. Figure 4.3e. shows that the TSC could be maximized to 213.46 g/L at about 30 min and 1:10 g/mL solid-to-liquid ratio over a range of the other operational factors (temperature and shaking speed). In fact, in water bath extraction, shaking speed is one of the key variables affecting the release of sugars from different matrices. An increase in the shaking speed accelerated sugar extraction. Figure 4.3f. shows that with an increase in shaking speed from 70 to 110 rpm and solid-to-liquid ratio from 1:7 to 1:10 g/mL, the extraction yield increased gradually from 186.67 to 229.75 g/L.

From the model, the optimal conditions for the conventional extraction (CE) were: temperature 90 °C, extraction time 55.34 min, shaking speed 110 rpm and solid-to-liquid ratio 1:10 g/mL with a predicted yield of 233.54±5.41 g/L. The extraction was carried out under these conditions giving a real recovery of 234.38±2.43 g/L.

4.2.4 Verification of the predictive models

Based on the above findings, to validate the predictability of the established models, the optimized parameters were tested. The mean values 233.25±3.59, 202.89±5.80 and 233.54±5.41 g/L were found in the actual experiments for the MAE, UAE and CE, respectively, which were in close agreement with the predicted values (233.80±1.90, 202.04±3.40 and 234.38±2.43 g/L). The strong semilarity between these results confirmed that the response models were adequate in predicting the optimized conditions. The empirical relationship between the extraction yield of date sugars and the independent variables, expressed by a quadratic model, were given in the following equations.

$$Y_{MAE}\ (TSC) = 222.59 + 3.16X_1 - 0.79X_2 + 18.52X_3 + 1.51X_1X_2 + 1.73X_1X_3 + 0.49X_2X_3 - 16.53X_1^2 - 2.39X_2^2 - 8.23X_3^2 \tag{4.2}$$

$$Y_{UAE}\ (TSC) = 179.42 + 10.03X_1 - 1.06X_2 + 15.11X_3 + 0.30X_1X_2 - 2.28X_1X_3 + 1.94X_2X_3 + 2.87X_1^2 - 2.23X_2^2 - 2.41X_3^2 \tag{4.3}$$

$$Y_{CE}\ (TSC) = 201.52 + 17.03X_1 - 1.66X_2 + 5.38X_3 + 16.06X_4 - 2.04X_1X_2 - 2.35X_1X_3 - 0.65X_1X_4 - 5.77X_2X_3 + 1.42X_2X_4 + 0.74X_3X_4 - 4.18X_1^2 - 9.40X_2^2 - 0.85X_3^2 + 5.73X_4^2 \tag{4.4}$$

Where Y is the extraction yield of date total sugars and X_1, X_2, X_3 and X_4 are the uncoded variables.

The exploration and the optimization of a fitted response surface may produce poor or misleading results unless the model exhibits a good fit, which makes it essential to verify the model's adequacy (Bimakr et al., 2012). The statistical analysis was performed to estimate the multiple factor effects on the performance of the treatment by the input variables. The statistical analysis indicates that the P-values ($<$0.0008) indicate model terms are significant. Values greater than 0.05 indicate the model terms are not significant. F-test for lack of fit explains the difference of data for the model (Singh et al., 2019a). Higher F-values indicate a greater spread of data around the model, indicating the model's suitability for high variations (Singh et al., 2019b). Table 4.3 shows the results of the second-order response surfaces in the form of analysis of variance (ANOVA). The significance of each coefficient was determined by the F-test and the p-value. The corresponding variables would be more significant if the absolute F-value becomes greater and the p-value becomes smaller (Kamairudin et al., 2021).

The independent variables: microwave power (X_1), irradiation time (X_2) and solid-to-liquid ratio (X_3) in the MAE, ultrasonic temperature (X_1), ultrasonic treatment time (X_2) and solid-to-liquid ratio (X_3) in the UAE, and temperature (X_1), treatment time (X_2), solid-to-liquid ratio (X_3) and shaking speed (X_4) in the WBE, were the linear terms. The results suggest that the change in these variables have a highly significant effect on the total sugar content (TSC) ($p<0.0001$), when the microwave-assisted, ultrasound-assisted and conventional extractions were used. The non-significant value of the lack of fit (more than 0.05) showed that the quadratic models were valid for the present study. The Fisher's F-values (4040.3, 2751.5 and 8151.5) with a very low probability values ($p<0.0001$) implied that the models were highly significant. The goodness of fit of the models were examined by the determination coefficients (${R_1}^2$=0.9940, ${R_2}^2$=0.9910 and ${R_3}^2$=0.9930) for the microwave-assisted, ultrasound-assisted and conventional extractions, respectively, which implied that the samples variation (99.40, 99.10 and 99.30 %) were statistically significant and only 0.06, 0.09 and 0.07 %, respectively, of the total variance could not be explained by the model. The adjusted determination coefficients (${R_{Adj_1}}^2$=0.9831, ${R_{Adj_2}}^2$=0.9747 and ${R_{Adj_3}}^2$=0.9849) and the predicted determination coefficients (${R_{Pred_1}}^2$=0.8881, ${R_{Pred_2}}^2$=0.9448 and ${R_{Pred_3}}^2$=0.9035) were also satisfactory to confirm the significance of the models.

Table 4.3. Analysis of variance (ANOVA) for the experimental results of MAE, UAE and CE methods.

MAE				**UAE**			
	Sum of squares	***F*-value**	***p*-value**		**Sum of squares**	***F*-value**	***p*-value**
Model	4040.3	91.4	< 0.0001	Model	2751.5	60.89	< 0.0001
X_1	3.16	4.04	0.0099	X_1	10.03	12.66	< 0.0001
X_2	-0.79	-1.00	0.3613	X_2	-1.06	-1.43	0.2368
X_3	18.52	23.63	< 0.0001	X_3	15,11	19.07	< 0.0001
X_1X_2	1.51	1.36	0.2305	X_1X_2	0.31	0.28	0.79
X_1X_2	1.72	1.56	0.1795	X_1X_3	-2.28	-2.04	0.10
X_2X_3	0.49	0.45	0.6744	X_2X_3	1.94	1.74	0.14
${X_1}^2$	-16.53	-14.33	< 0.0001	${X_1}^2$	2.87	2.46	0.06
${X_2}^2$	-2.39	-2.07	0.0928	${X_2}^2$	-2.23	-1.91	0.11
${X_3}^2$	-8.23	-7.13	0.0008	${X_3}^2$	-2.41	-2.07	0.09
Residual	24.56			Residual	25.11		
Lack of Fit	91.40	1.30	0.46	Lack of Fit	60.89	1.07	0.52
Pure Error	8.32			Pure Error	9.64		
R^2	0.99			R^2	0.99		
${R^2}_{adj}$	0.98			${R^2}_{adj}$	0.97		
RMSE	2.22			RMSE	2.24		
Cor Total	24.56			Cor Total	25.11		
CE							
	Sum of squares	***F*-value**	***p*-value**				
Model	8151.5	122	< 0.0001				
X_1	17.03	26.9	< 0.0001				
X_2	-1.66	-2.63	0.0221				
X_3	5.38	8.53	< 0.0001				
X_4	16.06	25.5	< 0.0001				
X_1X_2	-2.04	-1.86	0.0869				
X_1X_3	-2.35	-2.15	0.0529				
X_1X_4	-5.77	-0.59	0.5641				
X_2X_3	-0.65	-5.28	0.0002				
X_2X_4	1.42	1.30	0.2183				
X_3X_4	0.74	0.68	0.5106				
${X_1}^2$	-4.18	-4.42	0.0008				
${X_2}^2$	-9.40	-9.93	< 0.0001				
${X_3}^2$	-0.85	-0.90	0.0008				
${X_4}^2$	5.73	6.05	< 0.0001				
Residual	57.29						
Lack of Fit	121.95	0.40	0.87				
Pure Error	19.03						
R^2	0.99						
${R^2}_{adj}$	0.98						
RMSE	2.19						
Cor Total	8208.8						

4.2.5 Comparative study of the extraction processes

Date syrups were made from date juices extracted by three methods. The green technologies, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), were compared with each others and with the conventional extraction(CE) considering the total sugar content (TSC). A set of samples were extracted under optimum conditions, and the predicted results fitted well with the experimental results: 233.80 ± 1.90^a g/L for MAE, 202.04 ± 3.40^b g/L for UAE and 234.38 ± 2.43^a g/L for CE.

The selection of an extraction method would mainly depend on the advantages and disadvantages of the processes such as the extraction yield, complexity, production cost, environmental friendliness and safety (Li et al., 2012). The results indicated that the MAE showed a similar extraction capacity as compared to the CE ($p>0.05$). However, the extracted TSC by UAE was significantly lower than that of MAE and CE ($p<0.05$). This results were not in agreement with that reported by Ganbi (2012), who found the highest total sugar content (88.94%) in the syrup extracted by ultrasonic procedure. The yield using the microwave-assisted extraction greatly reduced the processing time, the shortest processing time (1.99 min) occurred at 530 W microwave power. However, conventional extraction took the same time as ultrasound-assisted extraction, about 60 min. Microwave-assisted extraction was the most efficient method for extracting sugars based on the lower extraction time.

4.2.6 Identification of sugars from date syrups by HPLC-RID

Carbohydrates are the major constituents of date fruits, including mainly reducing sugars such as glucose and fructose, and non-reducing sugars such as sucrose, and small amounts of polysaccharides such as cellulose (Ahmed et al., 2014). In the present study, sucrose, glucose and fructose were identified in date syrups obtained by microwave-assisted, ultrasound-assisted and water bath extraction methods (60.11, 16.64 and 23.25% for MAE, 00.00, 46.94 and 53.06% for UAE and 50.96, 20.67 and 28.55% for WBE, respectively) (Table 4.4). Concerning reducing sugars, all syrups contain more fructose than glucose, which is in agreement with previous study of Yahmed et al. (2021). High-fructose syrup is largely used as a sweetener in food and pharmaceutical industries, as well as to attain crystalline fructose. Moreover, compared to sucrose

syrup, it has more desirable functional properties, such as high solubility and it prevents the crystallization of sugar in food products (Amaral-Fonseca et al., 2021).

Table 4.4. Identification of individuel sugars in date syrups by HPLC-RID.

Extraction technique	**Sucrose (%)**	**Gulcose (%)**	**Fructose (%)**
Microwave-assisted extraction (MAE)	60.11	16.64	23.25
Ultrasound-assisted extraction (UAE)	00.00	46.94	53.06
Conventional extraction (CE)	50.76	20.67	28.55

Data are mean values ± standard deviation.

Fructose, sucrose and glucose, when present in aqueous solutions, activate sweet taste receptors present in the mouth, particularly on the tongue. The resulting pleasant sensation has been shown to be associated with activation of dopaminergic reward pathways located in the mesolimbic nervous system. This positive hedonic response to sugars is thought to have developed as a means of promoting the consumption of energy-dense, sugary foods (Tappy and Rosset, 2017). Glucose and fructose, monomers of reducing sugars, were present in the three date syrups. They are the product of the enzymatic reaction of invertase on disaccharides. This enzyme hydrolyses sucrose into glucose and fructose completely in soft date cultivars, and partially in semi-dry and dry date cultivars (Ganbi, 2012). Indeed, the increase in invertase activity is the main factor for reducing sugar increase, and a direct correlation was found between the reducing sugar content of dates and the invertase activities (Chaira et al., 2011).

As we can see in both syrups obtained by microwave-assisted and conventional or water bath extractions (MAE and CE or WBE), the major sugars were the non-reducing one (sucrose). Indeed, the dry dates contain relatively more sucrose than reducing sugars, which confers a very pleasant taste to the fruit (Mrabet et al., 2008). It was also worth high lighting the absence of sucrose in the syrup obtained by ultrasound-assisted extraction (UAE), which could be explained by the increases of invertase activity, during the ultrasound treatment. It has been reported that sucrose hydrolysis by invertase is accelerated by ultrasound irradiation (Kwiatkowska et al., 2011). On the other hand, the reducing sugar release, in cassava chip slurry, was as high as 180% of the control samples and the slurry samples with enzyme addition during sonication resulted in greater release than samples with enzyme addition after sonication (Nitayavardhana et al., 2008).

4.3 Conclusion

In this study, green extraction technologies, microwave-assisted and ultrasound-assisted extractions (UAE and MAE), were compared with a conventional one (CE) for the extraction of total sugars from Degla-Beida date variety. The response surface methodology (RSM) was successfully applied to optimize the three extraction methods. Microwave power, ultrasonic temperature, water bath temperature, extraction time, solid-to-liquid ratio and shaking speed played a significant role in the extraction of the investigated compounds. We were able to extract higher yields of the constituent from *Phoenix dactylifera* L., while using microwave-assisted extraction, which simultaneously reduced the extraction time considerably compared with two other methods that take along extraction time. However, if we consider the nature of the sugars extracted by these three methods and analyzed by HPLC-RID, the ultrasound technique was the one that gives the best yields of reducing sugars and especially fructose, which is known for its desirable functional properties.

Chapter 5

Date syrup and powder as alternative sweeteners to sucrose in dairy dessert processing using the D-Optimal mixture design

The global market has increased attention to the solution of justification, development and supply chain management to world markets of promising functional foods, recognizing it as the most important factor in the transfer of the various population groups to a healthy lifestyle, including healthy diet. Date syrup and powder have been fully considered as a potential functional food ingredients to develop foods with promising health benefits. Based on the available information in the literature, they are rich in phytochemicals. In addition, they were reported to possess several beneficial health properties explored under *in vitro* and *in vivo* conditions. According to the goal set in this work, the following tasks were addressed: 1) Formulation of dairy desserts: syrup dairy dessert (SDD), powder dairy dessert (PDD) and mixture dairy dessert (MDD), using the D-optimal mixture design. 2) Determination of the optimized mixture proportions of date syrup (DS) and date powder (DP), for the best mixture dairy dessert formulation, to achieve the desired sensory properties for effective formulation process. 3) Investigation of the possible influence of this incorporation on the sensory, physicochemical, phytochemical, microbiological, rheological and microstructural characteristics, with a comparison to a commercial dairy dessert (CDD).

5.1 Materiel and methods

5.1.1 Dairy dessert processing

After prior preparation of date syrup and powder as described by Djaoud et al. (2019), the dairy desserts were formulated following the optimal technological conditions found by Hamza-Chaffai (1990). Briefly, in a container, the milk, which is the basic ingredient, with different proportions of sugar, date syrup and powder (Table 5.1.), starch and carrageenan were mixed. The ingredients were homogenized in a shaking water bath, packaged in jars, cooled and stored in a refrigerator at 4 °C (Djaoud et al., 2020a).

5.1.2 Experimental design

The D-optimal mixture design, compared to other designs, is preferable for the optimization of ingredient mixtures to produce desirable responses (Chouhan and Saini, 2016). In this study, a three-factor, two-level D-optimal mixture design, generated by the statistical software package JMP (10.0.0 version, SAS Institute, USA), was employed to construct polynomial models for the optimization process. The selected factors were: X_1–Date syrup, X_2–Date powder and X_3–Sugar, whereas the responses were the following sensory descriptors: Y_1–Texture, Y_2–Hardness, Y_3–Granularity, Y_4–Sweetness, Y_5–Color, Y_6–Odor and Y_7–Savor. Table 5.1, shows the coded variables of the six formulations suggested by the Design-Expert® software prepared with different concentrations of date syrup, date powder and sugar, respectively: MDD_1 (0.125–0.875–0), MDD_2 (0.125–0.125–0.75), MDD_3 (0.125–0.125–0.75), MDD_4 (0.125–0.875–0), MDD_5 (0.875–0.125–0) and MDD_6 (0.875–0.125–0). In addition, syrup dairy dessert SDD (1–0–0) and powder dairy dessert PDD (0–1–0) were evaluated.

Table 5.1. Coded variables of dairy dessert formulations.

Sample	X_1-Date syrup	X_2-Date powder	X_3-Sugar
MDD_1	0.125	0.875	0
MDD_2	0.125	0.125	0.750
MDD_3	0.125	0.125	0.750
MDD_4	0.125	0.875	0
MDD_5	0.875	0.125	0
MDD_6	0.875	0.125	0
SDD	1	0	0
PDD	0	1	0

5.1.3 Sensory evaluation

As mentioned by Wang et al. (2018), ten panelists (n=10; including 5 women and 5 men) familiar with sensory evaluation techniques and regular consumers of dairy desserts evaluated the sensory quality of the samples. The panelists, were presented with 6 coded samples (40 g) developed with different concentrations of date syrup, date powder and sugar, respectively, according to the combinations generating by the mixture design: 2–14–0% (MDD_1), 2–2–12% (MDD_2), 2–2–12% (MDD_3), 2–14–0% (MDD_4), 14–2–0% (MDD_5), and 14–2–0% (MDD_6), and two other formulations developed with date syrup 16–0–0% (SDD) and date powder 0–16–0% (PDD), using a five–point hedonic scale, ranging from 5 (extremely liked) to 1 (extremely disliked), for each sensory attribute: texture, hardness, granularity, sweetness, color, odor, flavor and savor with an evaluation of overall acceptability on a scale of 1 to 9 (Annex 2).

5.1.4 Physicochemical analyses

5.1.4.1 Water content

The water content of the dairy desserts (CDD, SDD and MDD) was determined as described by Boulekbache et al. (2013) (Section 3.1.3.1).

5.1.4.2 Protein content

The protein content of the samples was determined as % N $\times$ 6.38 using a LECO CHNS-932 nitrogen microanalyser (Leco Corporation, St Joseph, USA) (da Silva et al., 2019). The process consists of burning the protein isolate (950-11000 °C) to convert nitrogen (N) to dinitrogen (N_2) in order to obtain a quantitative result. Subsequently, the generated N_2 was quantified using a micro analyzer (IrDA: Infrared Data Association).

5.1.4.3 Lipid content

The lipids of each sample were extracted in a Soxhlet apparatus using hexane as a solvent, boiling for 30 min (Alem et al., 2017). A flask containing about 2 g of sample (packed in a Wattman paper filter "W_1") was placed in a Soxhlet extractor under heating. The solvent was introduced through the condenser, attached to the Soxhlet equipped with a cooling system, which allows lipids to be extracted by a continuous

reflux process and then to be concentrated. The sample and the filter paper were cooled in a desiccator, dried until the solvent evaporated completely and reweighed "W_2". The lipid content was determined according to the equation (5.1).

$$\text{Lipid content (\%)} = [(W_1 \ / \ W_2) \ / \ W_1 \ ] \times 100 \tag{5.1}$$

5.1.4.4 Sugar content

As for sugars, the content of the formulated desserts was deduced from those of syrup and powder, determined in our study using the HPLC-RID (Djaoud et al., 2019). However, that of commercial product (CDD) was fixed by the company Soummam Dairy (company-specific standard).

5.1.4.5 Electrophoresis SDS–PAGE

To confirm the modification (*i.e.* cross-linking) of milk proteins in the syrup dairy dessert (SDD) and the optimal formulation of mixture dairy dessert (MDD) in comparison with a commercial dairy dessert (CDD), a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was performed as described by Laemmli (1970). A mini Protein Electrophoresis Cell (Bio-Rad Laboratories, California, USA) and a discontinuous system composed by a stacking gel (4 g/100 g) and a separating gel (10 g/100 g) were used for the identification of the cross-linking proteins. After the run, the gel was stained with the dye Coomassie Blue R–250 (1.92 g) in a mixture of methanol (40 mL), glacial acetic acid (160 mL) and distilled water (400 mL). For the decolorization of the gel, a solution containing methanol (200 mL), ethanol (100 mL), glacial acetic acid (50 mL) and distilled water (300 mL) were prepared. The protein fractions were identified using the molecular weight standard from 6400 to 200 000 $g.mol^{-1}$ (Natural Unstained Standards-Large gamme, Bio-Rad).

5.1.5 Total phenolic content (TPC) and antioxidant activity

As described by Amerinasab et al. (2015), 10 mg of each dairy dessert sample were extracted with 10 mL of distilled water using a stir plate. The mixture was centrifuged

(NF200 NÜVE, Turkey) at 4000 rpm for 20 minutes and the supernatant was thus collected. The suspension was filtered, the filtrate was used as a stock solution for the analysis of phenolic compounds as described by Georgé et al. (2005) (Section 3.1.4.2).

The ability of the extracts to scavenge the DPPH free radicals was determined following the method used by Milardović et al. (2006) (Section 3.1.5.1). However, the reducing power was determined according to Oyaizu (1986) method (Section 3.1.5.3).

5.1.6 Microbiological analysis

The count of the total flora and the search for undesirable microorganisms (coliforms, fecal coliforms and *Staphylococcus aureus*) were performed according to the standard procedure (JORA, 2017). Total flora was determined using plate count agar with an incubation at 32 °C for 48 h. Presumptive coliforms were enumerated on purple red bile agar, after incubation at 37 °C for 24 h in the case of total coliforms and 44 °C for 72 h in the case of fecal coliforms. *Staphylococcus aureus* were determined using the Baird–Parker agar supplemented with tellurite, incubated at 35 °C for 48 h.

5.1.7 Rheological measurements

The rheological measurements of the commercial dairy dessert (CDD), syrup dairy dessert (SDD), and optimal formulation of mixture dairy dessert (MDD) were performed in a controlled-stress rheometer (AR2000, TA Instruments USA) using a parallel plate geometry with a rough surface (60 mm diameter; 1 mm gap), in order to avoid slipping effects (Zarzycki et al., 2019). The measurements were taken at 10±1 °C, selected as a representative temperature of the usual consumption of dairy desserts. The temperature was kept constant by means of a circulating water bath. The samples were placed between the cone and the plate and the edges were removed. Each lot was measured in triplicate, using a fresh sample for each measurement.

5.1.7.1 Steady-state shear measurements

Steady-state shear flow test were performed from 1 to 200 s^{-1} for the three samples. The results were adjusted to the Ostwald-de Waele model, which is one of the methods known for explaining the behaviour of fluids.

The model applies the following expression:

$$\eta = K\gamma^{n-1} \tag{5.2}$$

Where η is the apparent viscosity, γ is the shear rate, K and n are the consistency and flow index, respectively. The apparent viscosity (η) at intermediate shear rate (γ = 0.01 s^{-1}) and flow index (n) were the parameters selected for comparison. The flow index (n) is selected because it determines how the flow precisely develops, if n<1 the fluids called pseudoplastic, these fluids flow more easily by increasing shear rate (shear–thinning). On the other hand, when n>1, the flow resistance increases with increased shear rate, and it is called a dilatant fluid (shear-thickening). In order to compare, viscosity values at 10 s^{-1} (η_{10}) were selected.

5.1.7.2 Viscoelastic behaviour

Stress sweep tests were made between 0.2 and 300 Pa, at a frequency of 1 Hz (6.28 rad/s), in all the systems studied to determine the linear viscoelasticity region and the critical stress. Frequency sweeps tests at 0.05 Pa, which is within the linear viscoelastic region since this value (0.05 Pa) is lower than the critical stresses for all the systems, were performed from 0.01 to 100 rad/s. Storage and loss moduli (G' and G", respectively) were plotted as a function of frequency. The oscillatory rheological parameters used to compare the viscoelastic properties of the samples were storage modulus (G') and loss tangent (tan δ=G"/G') at 2 rad/s (G'_2 and tan δ_2).

5.1.8 Confocal laser scanning microscopy (CLSM)

The microstructure of the commercial dairy dessert (CDD), syrup dairy dessert (SDD), and optimal formulation of mixture dairy dessert (MDD) was observed using a confocal laser scanning microscopy (CLSM) in a reflectance mode, as described by Kermiche et al. (2018), using a ZEISS LSM7 DUO (Germany) microscopy, with an Argon laser at 488 nm and detection between 493 and 619 nm. The microstructure images were analyzed using image analysis software (ZEN2012SP1).

5.1.9 Statistical analysis

The data have been reported as means ± standard deviation (SD), statistically assessed by ANOVA analysis of variance and Tukey's post hoc test with 95% confidence level, using XLSTAT Release 10 (Addinsoft, Paris, France).

5.2 Resultats et discussion

5.2.1 Analysis and validation of the D-optimal mixture design

A three-factor, two-levels, D-optimal mixture design was used in this study to correlate the effect of the formulation ingredients (X_1-Date syrup, X_2-Date powder and X_3-Sugar) with the observed responses (Y_1-Texture, Y_2-Hardness, Y_3-Granularity, Y_4-Sweetness, Y_5-Color, Y_6-Odor and Y_7-Savor). The lead number of experiments (n=6) was suggested by the Design-Expert® software, and runs were randomly set. The independent and dependent design variables were listed in Table 5.1 with the experimental runs. After producing and analyzing the formulations, sensory responses were applied to fit the appropriate model (linear). The model was tested for goodness of fit (R^2), and analysis of variance (ANOVA) was applied to verify the adequacy of the regression model in terms of a lack-of-fit test. This test implies that the residual response sum of squares is separated into the components model error and pure error, and their significances were obtained by an F-test (Table 5.2). The probability value (p-value) for the determination of the statistical significance was set at 0.05, which indicates that an "hypothesis" theory would be rejected if the calculated p-value was less than 0.05 in favor of an alternative theory. As seen in Table 5.2, the linear model was statistically significant (p: 0.0316, 0.0020, 0.0011, 0.0011, 0.0023, 0.0200 and 0.0025, respectively), which indicates that the model adequately describes the responses. The lack of fit test was subsequently performed to further demonstrate the suitability of a given model. Lack-of-fit test, which was calculated based on the residual sum of squares, diagnoses whether a model adequately fits the data. Insignificant lack of fit values of the linear models (p>0.05) for Y_1 (0.36), Y_2 (0.44), Y_3 (0.27), Y_4 (0.42), Y_5 (0.13), Y_6 (0.26) and Y_7 (0.42), respectively, indicates that the model adequately fits the data.

Table 5.2. Analysis of variance (ANOVA) for the regression model.

Source	**Sum of squares**	**DOF**	**Mean square**	***F*-value**	***p*-value**	**Sum of squares**	**DOF**	**Mean square**	***F*-value**	***p*-value**
	Y_1-Texture					**Y_1-Hardness**				
Model	0.63	2	0.32	13.50	0.0316	8.90	2	4.45	92.10	0.0020
X_1	35.28	1		1512.00	<0.0001	38.72	1		801.10	<0.0001
X_2	23.81	1		1020.21	<0.0001	5.78	1		119.59	0.0016
X_3	32.81	1		1405.93	<0.0001	34.45	1		712.61	0.0001
Lack of Fit	0.01	1	0.01	1.15	0.73	0.05	1	0.05	0.90	0.44
Residual	0.07	3	0.02			0.15	3	0.05		
Pure Error	0.07	1	0.03			0.10	2	0.05		
R^2	0.90					0.98				
$R^2{}_{adj}$	0.83					0.97				
Cor Total	0.70	5				9.05	5			
	Y_3-Granularity					**Y_4-Sweetness**				
Model	8.04	2	4.02	141.94	0.0011	5.45	2	2.73	136.33	0.0011
X_1	28.88	1		1019.23	<0.0001	38.72	1			<0.0001
X_2	5.12	1		180.71	0.0009	8.82	1			0.0002
X_3	36.13	1		1275.00	<0.0001	25.92	1			<0.0001
Lack of Fit	0.05	1	0.05	0.25	0.27	0.02	1	0.02	1.00	0.42
Residual	0.09	3	0.03			0.06	3	0.02		
Pure Error	0.04	2	0.02			0.04	2	0.02		
R^2	0.99					0.99				
$R^2{}_{adj}$	0.98					0.98				
Cor Total	8.13	5				5.51	5			
	Y_5-Color					**Y_6-Odor**				
Model	9.26	2	4.63	84.21	0.0023	1.82	2	0.91	18.86	0.0200
X_1	42.32	1		769.45	0.0001	32.00	1		662.07	0.0001
X_2	33.62	1		611.27	0.0001	14.05	1		290.59	0.0004
X_3	6.13	1		111.36	0.0018	21.78	1		450.62	0.0002
Lack of Fit	0.13	1	0.13	6.25	0.13	0.08	1	0.08	2.46	0.26
Residual	0.17	3	0.06			0.15	3	0.05		
Pure Error	0.04	2	0.02			0.07	2	0.03		
R^2	0.98					0.93				
$R^2{}_{adj}$	0.97					0.88				
Cor Total	9.43	5				1.97	5			
	Y_7-Savor									
Model	7.24	2	3.62	80.44	0.0025					
X_1	36.13	1		802.78	<0.0001					
X_2	5.45	1		121.00	0.0016					
X_3	25.21	1		560.11	0.0002					
Lack of Fit	0.05	2	0.05	1	0.42					
Residual	0.14	3	0.05							
Pure Error	0.09	2	0.05							
R^2	0.98									
$R^2{}_{adj}$	0.97									
Cor Total	7.38	5								

The values of R^2 (0.90, 0.98, 0.99, 0.99, 0.98, 0.93 and 0.98) and adjusted R^2 (0.83, 0.97, 0.98, 0.98, 0.97, 0.98 and 0.97) indicated that the experimental data were in a good agreement with predicted values. Considering the data shown in Table 5.2, mathematical predictive models of mixture ratio of date syrup, date powder and sugar on the sensory characteristics of the dairy dessert formulations can be developed. According to the equations (5.3), (5.4), (5.5), (5.6), (5.7), (5.8) and (5.9), the mathematical predictive models were a first-order regression model, with no interaction term, so the studied factors have a significant impact to the formulation of dairy desserts, and X_1 (*i.e.*, date syrup) was the most significant factor.

The effect of the independent variables on the sensory characteristics of the dairy dessert formulations can be implemented by a triangular-dimensional contour plot. Figure 5.1 describe the variation on the responses as a function of the mixture composition, and clearly indicates that the sensory characteristics enhance mainly by an increased ratio of date syrup (X_1). A lower quantity of date powder and sugar (X_2 and X_3) was better for the sensory quality of the mixture dairy dessert formulations. The optimum mixture dairy dessert could provide more nutrition for consumers with the total substitution of sugar by date syrup and powder.

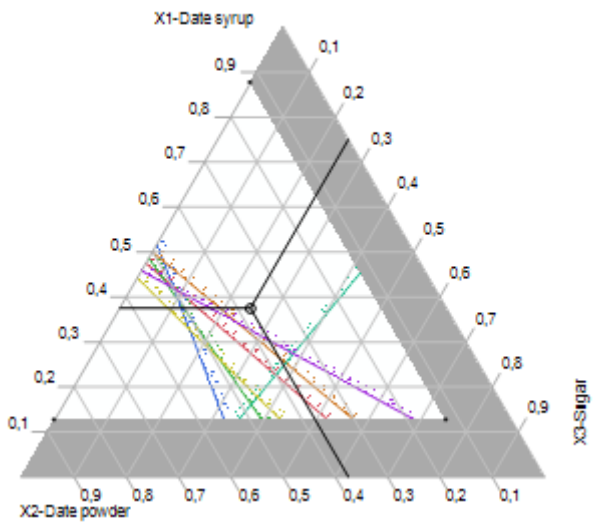


Figure 5.1. Triangular-dimensional contour plot illustrating the effect of date syrup (X_1), date powder (X_2) and sugar (X_3) on the responses.

Additional experiments with optimal conditions (X_1-0.875, X_2-0.125 and X_3-0) were carried out in order to assess the validity of the model. The experimental values (4, 4.6, 3.8, 4.5, 4.9, 4.3 and 4.3) were found to be reasonably close to the predicted ones (4.2, 4.4, 3.8, 4.4, 4.6, 4, and 4.25), which confirmed the validity and adequacy of the predicted models. Obviously, these results confirms the excellent reproducibility of the experimental conclusion. The mathematical predictive models are as follows:

$$Y_1(\text{Texture}) = 4.2X_1 + 3.45X_2 + 4.05X_3 \tag{5.3}$$

$$Y_2(\text{Hardness}) = 4.4X_1 + 1.7X_2 + 4.15X_3 \tag{5.4}$$

$$Y_3(\text{Granularity}) = 3.8X_1 + 1.6X_2 + 4.25X_3 \tag{5.5}$$

$$Y_4(\text{Sweetness}) = 4.4X_1 + 2.1X_2 + 3.6X_3 \tag{5.6}$$

$$Y_5(\text{Color}) = 4.6X_1 + 4.1X_2 + 1.75X_3 \tag{5.7}$$

$$Y_6(\text{Odor}) = 4X_1 + 2.65X_2 + 3.3X_3 \tag{5.8}$$

$$Y_7(\text{Savor}) = 4.25X_1 + 1.65X_2 + 3.55X_3 \tag{5.9}$$

Where X_1, X_2 and X_3 denoted date syrup, date powder and sugar, respectively.

5.2.2 Sensory evaluation

Understanding how consumers perceive dairy foods is critical for food industry. This information is essential for the development and marketing of new products, the reformulation of existing ones, and the optimization of manufacturing processes. Apart from knowing how much consumers like a product, in order to design food products that meet consumer sensory expectations, information about which sensory characteristics consumers expect to find in the product are needed, *i.e.*, which sensory attributes drive consumer liking. As consumers are not always able to say why they like a product, affective, descriptive and projective sensory methods have been widely used in several studies to answer this question (Pinto et al., 2018; Torres et al., 2017).

Photography 5.1. Dairy desserts with date syrup and powder (Degla-Beida).

Consumers' affective tests, such as the test run in this study (Photography 5.1), provide useful information on the future commercial potential of a new developed food. Table 5.3 shows the means sensory ratings for the dairy desserts, ranged from extremely disliked to extremely liked (from 1 to 5, respectively), containing varying percentages of date syrup, date powder and sugar respectively, 2–14–0% (MDD_1), 2–2–12% (MDD_2), 2–2–12% (MDD_3), 2–14–0% (MDD_4), 14–2–0% (MDD_5), 14–2–0% (MDD_6), 16–0–0% (SDD) and 0–16–0% (PDD).

There were a significant differences in texture, hardness, granularity, sweetness, color, odor, savor and overall acceptability between the samples. The minimum scores were attributed to powder dairy dessert (PDD) which might be due to the high content of date powder (16%) providing intense astringency taste. However, syrup dairy dessert (SDD) was the most appreciated by consumers. Jridi et al. (2015) find that an increase in the quantity of syrup leads to reduce the astringent appearance in the final product.

Table 5.3. Observed responses for the three-component D-optimal mixture design.

	Texture	**Hardness**	**Granularity**	**Sweetness**	**Color**	**Odor**	**Savor**	**Acceptability**
MDD$_1$	3.60±0.84^{bc}	1.90±0.32^{b}	1.70±0.48^{b}	2.20±0.63^{c}	4.20±0.42^{a}	2.80±0.32^{c}	1.80±0.42^{c}	1.80±0.63^{b}
MDD$_2$	4.10±0.88^{ab}	4.30±0.48^{a}	4.40±0.70^{a}	3.70±0.82^{b}	2.00±0.48^{c}	3.50±0.71^{bc}	3.70±0.82^{ab}	6.90±0.99^{a}
MDD$_3$	4.00±0.15^{ab}	4.00±0.40^{a}	4.10±0.61^{a}	3.50±0.42^{b}	1.50±0.30^{c}	3.10±0.52^{ab}	3.40±0.46^{ab}	6.70±0.72^{a}
MDD$_4$	3.30±0.52^{bc}	1.50±0.35^{b}	1.50±0.12^{b}	2.00±0.28^{c}	4.00±0.37^{a}	2.50±0.10^{c}	1.50±0.15^{c}	1.50±0.18^{b}
MDD$_5$	4.30±0.20^{ab}	4.50±0.73^{a}	3.90±0.59^{a}	4.50±0.48^{a}	4.70±0.42^{a}	4.10±0.88^{ab}	4.40±0.31^{a}	7.10±0.58^{a}
MDD$_6$	3.10±0.88^{ab}	4.30±0.48^{a}	3.70±0.82^{a}	4.30±0.70^{a}	4.50±0.48^{a}	3.90±0.28^{bc}	4.10±0.15^{a}	7.20±0.95^{a}
SDD	4.80±0.42^{a}	4.60±0.52^{a}	4.60±0.52^{a}	4.80±0.42^{a}	4.60±0.52^{a}	4.60±0.70^{a}	4.40±0.97^{a}	7.60±0.84^{a}
PDD	3.10±0.32^{c}	1.70±0.48^{b}	1.50±0.53^{b}	2.30±0.95^{c}	3.60±0.70^{b}	1.70±0.48^{d}	1.60±0.70^{c}	1.60±0.52^{b}

Data are mean values ± standard deviation.
Values with letters (a-b-c) were significantly different (Tukey, $p<0.05$).

In addition, when comparing the mixture dairy dessert samples (MDD$_2$, MDD$_3$, MDD$_5$ and MDD$_6$) samples with 2% of date powder, which were the best scored compared to those containing 14% of date powder (MDD$_1$ and MDD$_4$). Regarding the data in Table 5.3, the panelists judged negatively the addition of date powder with high concentration (14%). In the case of date syrup and sugar, their use for the enrichment and sweeteness of dairy desserts improved the scores for all the descriptors.

This study demonstrates that the substitution of 16% sugars in dairy dessert by date syrup and powder as a suitable sweetener ingredients is possible. According to the results of mixture design, the best formulation of MDD was: 14% date syrup, 2% date powder and 0% sugar concentrations. Based on the obtained results, syrup dairy dessert SDD and optimal formulation of mixture dairy dessert MDD were selected to evaluate the possible influence of date syrup and powder (DS and DP) incorporation on their physicochemical, phytochemical, microbiological, rheological and microstructural characteristics, and the results were compared with a commercial dairy dessert CDD.

5.2.3 Physicochemical analyses

All foodstuffs deteriorate during storage, in particular those containing a product which is very sensitive to deterioration, namely milk. Deterioration in product quality may be the result of changing in the physicochemical factors.

5.2.3.1 Water content

The presence of huge available water quantities in the components is the principal factor inducing the development of bacteria, which affects safety and food quality. From Table 5.4, a significant difference ($p<0.05$) was observed between the water content of different dairy desserts: 77.84 ± 0.16^b, 76.98 ± 0.06^c and 78.48 ± 0.12^a% for syrup dairy dessert (SDD), optimal formulation of mixture dairy dessert (MDD), and commercial dairy dessert (CDD), respectively. Dairy desserts with date syrup and powder were characterized by the lowest water content compared to the commercial dairy dessert, this result could be explained by the greater richness of dates (Degla-Beida variety) in fibers. In fact, the presence of such components increased water retention in the corresponding product. Furthermore, among the two formulated samples, the highest level shown in syrup dairy dessert (SDD) might be due to the high proportion of syrup in this formulation. The incorporation of date syrup and powder into dairy desserts increased its dry matter. Indeed, the dry matter of the samples was: 22.16, 23.20 and 21.77% for syrup dairy dessert (SDD), optimal formulation of mixture dairy dessert (MDD, and commercial dairy dessert (CDD), respectively. Our data were in the range of those reported by Nongonierma et al. (2007) for yoghurts at 0 and 5% fat, added with sugar syrups (20.80 and 25.10%). They were also close to those obtained by Amellal (2008) for yogurts enriched with date powder of different Algerian common date varieties, between 20.64 and 21.39%.

5.2.3.2 Lipid content

Excess consumption of total and saturated fats is linked to the development of chronic diseases, such as obesity, heart disease, diabetes and cancer. There is therefore considerable interest in the development of foods containing lower levels of total and saturated fats, but that still have the same desirable physicochemical and sensory characteristics as the original foods (Iqbal et al., 2021).

Table 5.4 shows that the fat content of dairy desserts ranged between 1.44 and 2.16%, these values were almost identical for syrup dairy dessert SDD (2.16±0.13^a%) and the optimal formulation of mixture dairy dessert MDD (2.15±0.16^a%), compared to the commercial dairy dessert CDD (1.44±0.12^b%). These values were lower than those found by Medeiros et al. (2020) and de Sousa et al. (2021), which varies from 3.36 to 3.15% and 2.6 to 4.4%. On the other hand, they were in coincidence with those found by Amellal (2008), with values ranged from 2.12 to 3.95%. This is may be due to a difference in fat composition of milk and date fruits. The low lipid content means that the enriched dairy desserts show the best nutritional quality being healthier.

Table 5.4. Physicochemical analyses and antioxidant activity of dairy desserts.

Analysis	**CDD**	**SDD**	**MDD**
Water content (%)	78.48±0.12^a	77.84±0.16^b	76.98±0.06^c
Lipid content (%)	2.16±0.13^a	2.15±0.16^a	1.44±0.12^b
Protein content (%)	2.65±0.01^b	2.66±0.00^b	3.14±0.14^a
Sugar content (%)			
Sucrose	16.00±0.00^a	8.02±0.02^b	8.03±0.04^b
Glucose	0.00±0.00^c	3.95±0.01^b	3.87±0.02^a
Fructose	0.00±0.00^c	4.10±0.01^a	4.16±0.03^a
TPC (mg GAE/g DW)	0.27±0.00^c	0.65±0.04^a	0.38±0.03^b
DPPH (%)	2.54±0.46^b	4.00±0.82^a	3.22±0.44^{ab}
Reducing power (mg AAE/g DW)	0.17±0.16^c	2.75±0.10^a	2.21±0.17^b

Data are mean values ± standard deviation.

Values with different letters (a-b-c) were significantly (p<0.05).

5.2.3.3 Protein content

Plants have been used in the human diet as sources of energy and nutrients for many years. Plants protein market is growing rapidly as plants are seen as a sustainable solution to meet nutritional needs of the growing world population (Kaur et al., 2021). The protein content of the dairy desserts, detailed in Table 5.4, ranged from 2.65±0.01^b and 3.14±0.14^a%. The highest content was recorded for the optimal formulation of mixture dairy dessert (MDD) enriched with a mixture of 14% date syrup and 2% date powder (3.14±0.14^a%). These values were significantly lower (p<0.05) for syrup and commercial dairy desserts (2.66±0.01^b and 2.65±0.01^b%, respectively).

This result might be due to the incorporation of date powder to the mixture dairy dessert which enhanced its protein content. For a comparative purposes, we can say that this results agreed with those obtained by Jridi et al. (2015) who observed the enrichment of dairy desserts, in proteins, with date syrup and powder. These data were higher than those found by Medeiros et al. (2020) in non-fermented dairy desserts added with syrup and hydroethanolic extract derived from jabuticaba (*Myrciaria caulifora*) peels, and between the minimums and the maximums values reported by de Sousa et al. (2021), in dairy desserts containing *Caesalpinia pulcherrima* seed galactomannan, who reported values between 1.86-1.46 and 2.50-4.30%, respectively.

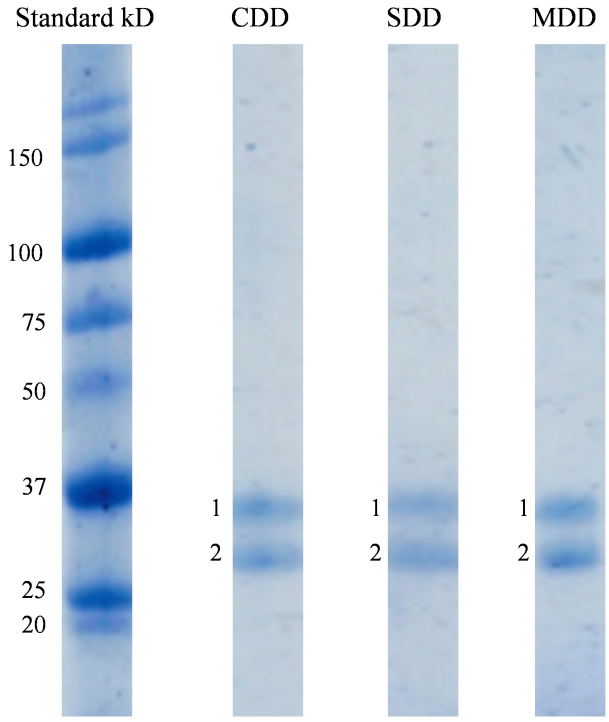
5.2.3.4 Sugar content

Table 5.4, represent the average contents of sucrose, glucose and fructose for each sample. The obtained results showed an extensive range of variation among the three sugars. The content of sucrose (8.02^b and 8.03^b%), glucose (3.95^b and 3.87^a%) and fructose (4.10^a and 4.16^a%) were found in syrup and mixture dairy desserts (SDD and MDD), respectively. The predominant sugar in all of the examined samples was determined as sucrose, followed by glucose and fructose compared to that of the commercial dairy dessert (CDD) sample, which was constituted by sucrose only (16%). To the best of our knowledge, few studies have been carried out on the individual sugar content of dairy desserts. Ahmed et al., (2016) have been reported that the total substitution of cacao by date powders, from H'lowa variety, in dairy dessert, increases the reducing sugars from 14.40 to 16.59%. These values were close to those obtained in our study.

5.2.3.5 Electrophoresis analysis

Typical electrophoregrams of dairy desserts (CDD, SDD and MDD) were shown in Figure 5.3. Two protein fractions were quantified at 29 and 32 kDa, which correspond to caseins β and α, respectively. No differences in the electrophoretic patterns were observed in the three samples, since repeatability of migration times was observed when we compared the commercial dairy dessert (CDD), syrup dairy dessert (SDD), and optimal formulation of mixture dairy dessert (MDD). These results show that the incorporation of date syrup and powder did not promote the appearance of new high molecular–weight protein bands. The absence of these bands indicate that no proteins

were formed by the cross–linking of low–molecular–weight polymers. This result was in accordance with that obtained by Oliveira et al. (2015), where caseins fraction had no apparent differences between white yoghurt and strawberry–enriched yoghurt.



Band	MD (kD)	Protein fraction
1	32	α-casein
2	29	β-casein

Figure 5.2. SDS-PAGE electrophoretograms of molecular weight markers as well as CDD, SDD and MDD protein fractions with an enclosed table of molecular weight (MW) and protein fraction name for each signal.

5.2.4 Total phenolic content (TPC) and antioxidant activity

Experimental studies have provided convincing evidence that food bioactive compounds have a positive biological impact on human health, exerting protective effects against noncommunicable diseases including cancer and cardiovascular, metabolic, and neurodegenerative disorders. These benefits have been associated with the presence of secondary metabolites, namely polyphenols, glucosinolates, carotenoids, terpenoids, alkaloids, saponins, vitamins, and fibres, among others, derived from their antioxidant, antiatherogenic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective, and vasodilator properties. Polyphenols as one of the most abundant classes of

bioactive compounds present in plant-based foods emerge as a promising approach for the development of efficacious preventive agents with reduced side effects (Câmara et al., 2021). So, for their effects on human health, many researches have been focused in their application mode in food industry. The incorporation of date syrup (DS) and date powder (DP) in dairy desserts (syrup dairy dessert (SDD) and mixture dairy dessert (MDD)), significantly improved their phenolic contents ($p<0.05$) compared with the commercial dairy dessert (CDD) by the enhancement of their antioxidant activity.

The results of total phenolic content (TPC) and antioxidant activity of dairy dessert samples were shown in Table 5.3. Syrup dairy dessert showed the highest total phenolic content. In fact, the addition of 16% of date syrup in SDD, and a mixture of 14% of date syrup and 2% of date powder in MDD, lead to an increase in the TPC with an amount of 0.65 ± 0.04^a and 0.38 ± 0.03^b mg GAE/g DW, respectively, compared with CDD (0.27 ± 0.00^c mg GAE/g DW). The increase of the phenolic content in the formulated dairy desserts was due to their enrichment with date by-products. Indeed, this fruit is a good source of bioactive molecules (Türker et al., 2020). The increase on the polyphenols concentration in dairy products enriched with various fruits has been reported in the literature (Amal et al., 2016; Kermiche et al., 2018).

The highest antioxidant activity was exhibited by syrup dairy dessert (SDD), with 4.00 ± 0.82^a% for the DPPH antiradical activity and 2.75 ± 0.10^a mg AAE/mL for the reducing power. However, the mixture dairy dessert (MDD) and commercial dairy dessert (CDD) showed a DPPH inhibition of 3.22 ± 0.44^{ab} and 2.54 ± 0.46^b%, and a reducing power of 2.21 ± 0.17^b and 0.17 ± 0.19^c mg AAE/g DW, respectively. Therefore, the addition of date syrup and powder in dairy desserts as a substitute for sugar (sucrose) led to a dairy dessert with high phenolic content and antioxidant activity.

These results were in agreement with those obtained by Ivanova et al. (2018), Jridi et al. (2015) and Szwajgier and Gustaw (2015), who observed the enrichment in total phenolic content and the enhancement of the antioxidant activity of dairy desserts adding with encapsulated cornelian cherry, chokeberry and black berry juices, date syrup and powder of different Tunisian varieties, and brewery malts, respectively.

Amerinasab et al. (2015), Trigueros et al. (2012) and Gad et al. (2010), noted the same thing in yoghurt based on date liquid sugar, yogurts manufactured with dates blanching water and yogurt resulting from combination of date palm syrup and skim milk, respectively. In the other hand, many studies have reported the enhancement of the antioxidant power of dairy products supplemented by fruits and vegetables (Felfoul et al., 2017; Kermiche et al., 2018; Sah et al., 2015).

5.2.5 Microbiological analysis

Dairy desserts are a popular dairy food, prepared with ingredients including milk as a basic constituent. They are palatable and nutritious, consumed by a wide range of people of all ages and are usually served cooled. Milk-based products are good media for the growth of microorganisms because of their high nutritive value (Amin, 2018). Therefore, thermal processing can afford a high degree of food security, but it causes thermal degradation in food quality including loss of flavor and nutrition quality. In this study, the formulated dairy desserts were heat-treated or pasteurized at 90 °C for 5 minutes. This treatment has a significant effect on the microbial flora as it destroys pathogenic and undesirable germs.

Table 5.5. Results of microbiological analysis of dairy desserts.

	Total aerobic flora	**Total coliforms**	**Fecal coliforms**	***Staphylococcus aureus***
SDD	< 1 CFU/g	Abs	Abs	Abs
MDD	< 1 CFU/g	Abs	Abs	Abs
CDD	< 1 CFU/g	Abs	Abs	Abs

According to the results of the microbiological analysis (Table 5.5), we distinguished values <1 CFU/g for the total aerobic flora, with an absence of total and fecal coliforms as well as *staphylococcus aureus* in the dairy desserts enriched with date syrup (SDD), the dairy dessert enriched with a mixture of date syrup and powder (MDD), and in the commercial dairy dessert (CDD). These results were in concordance with the statement of Mac Graw (1997), who reported that processed milk should contain no trace of coliforms. The absence of coliforms indicates adequate heating treatment of milk and high hygienic standards of processing and packaging that precluded recontamination of the product. These results confirm also the respect of aseptic conditions during the storage of the final product.

5.2.6 Rheological measurements

5.2.6.1 Steady-state shear properties

Relating to the steady-state rate profiles of the dairy desserts, shown in Figure 5.3, the samples exhibited a shear thinning behavior, *i.e.*, the shear stress increase and consequently the viscosity decrease with an increase in shear rate. In fact, the data curve, viscosity versus stress shear, of the samples were adequately fitted by the Ostwald-de Waele model (Türker et al., 2020). The consistency index (K) and the flow behavior index (n) of the dairy desserts were calculated and shown in Table 5.6.

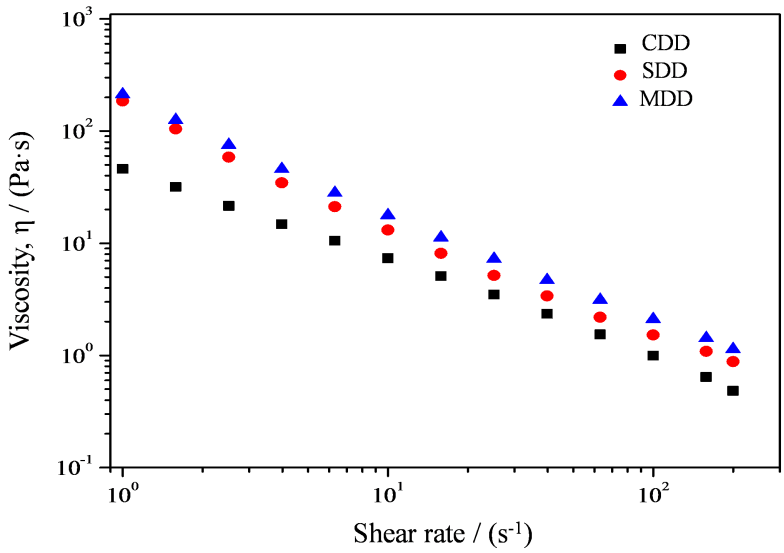


Figure 5.3. Steady shear flow curves (viscosity versus shear rate) of dairy desserts.

Although all the samples showed a shear thinning behavior, clear differences were observed among them, depending on the presence or not of date syrup and powder. It can be observed that syrup dairy dessert (SDD) and mixture dairy dessert (MDD) showed rather similarity in the flow curves unlike commercial dairy dessert (CDD), whose viscosity was clearly lower. In fact, these differences can be observed in η_{10} and K values showed in Table 5.6, where significant different values between these parameters (η_{10} and K) were found. The highest viscosity found in the mixture dairy dessert could be attributed to the presence of date powder. In addition, syrup and mixture dairy desserts were more sensitive to the shear rate since the flow index (n) was almost 0 in both cases (0.03). Therefore, the presence of date syrup and powder

lead to samples with higher viscosity but more sensitive under the effect of shear. These behaviors can be related to changes in the microstructure as a consequence of the components included by the date syrup and powder. The increase in viscosity is due to the complex composition of dates, including the presence of pectin (stabilizing agent) and mineral salts. Several authors have studied the effect of stabilizers on the thixotropic behavior of dairy products (Köksoy and Kılıç, 2003).

Table 5.6. Average values of apparent viscosity (η_{10}), consistency index (K), flow index (n), storage modulus (G'$_2$) and loss tangent (tan δ) of the dairy desserts.

	CDD	**SDD**	**MDD**
η_{10} **(Pa.s)**	7.34 ± 0.15^c	13.10 ± 0.80^b	17.70 ± 3.20^a
K (Pa.s)^n	49.13 ± 0.94^c	124.55 ± 11.80^b	194.53 ± 30.13^a
n	0.15 ± 0.01^a	0.03 ± 0.02^b	0.03 ± 0.02^b
G'$_2$ (Pa)	151.42 ± 19.12^b	1913.97 ± 62.93^a	1893.63 ± 45.43^a
tan δ_2	0.21 ± 0.00^a	0.18 ± 0.00^b	0.18 ± 0.00^b

Data are mean values ± standard deviation.

Values with different letters (a-b-c) were significantly ($p<0.05$).

5.2.6.2 Viscoelastic behaviour

Figure 5.4 shows the frequency sweeps corresponding to the commercial dairy dessert (CDD), syrup dairy dessert (SDD) and mixture dairy dessert (MDD).

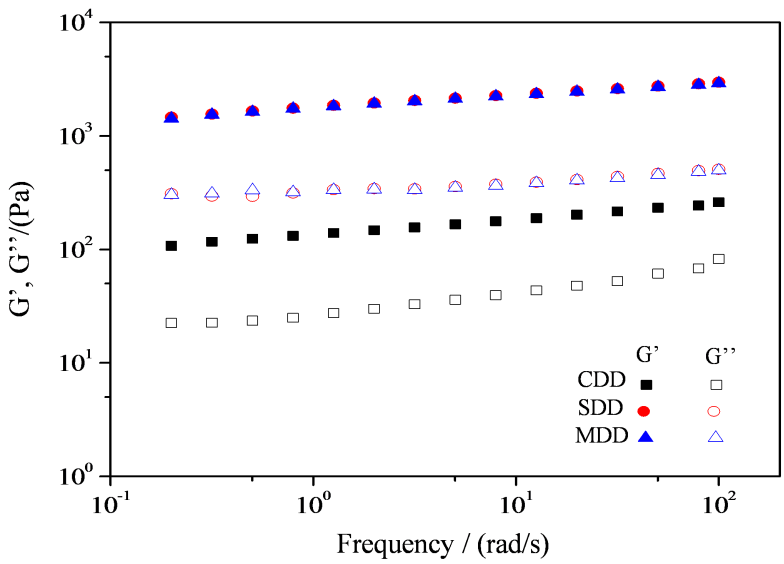


Figure 5.4. Mechanical spectra (G' and G" versus frequency) of dairy desserts.

The mechanical spectra of the samples correspond to a weak viscoelastic gel with a predominant elastic behavior, G' values were higher than G" ones, and both moduli showing slight variation with frequency (although a more dependence of G" values with frequency could be observed, showing the large contribution of the viscous component to higher frequencies). These results indicated that the interactions among ingredients influenced the rheological characteristics of the samples. In addition, syrup and mixture dairy desserts (SDD and MDD) were, at first sight, the ones with the strongest gel structure, G' and G" had a very similar values, indicating the similarity in the structure of the two samples. Once again, samples with date syrup and powder show a more structured system.

In fact, this behavior was proven by the highest values of G'_2 and the lowest tan δ_2 for syrup and mixture dairy desserts compared with the commercial dairy dessert, as shown in Table 5.6. These variations in dynamic parameters were due to the differences in the composition (pectin, sugar and mineral contents « Ca^{2+} precisely»). Moreover, Norziah et al. (2001), investigated the effect of pectin-sucrose-Ca^{2+} interactions on rheological properties. They found that increasing the concentration of Ca^{2+}, sucrose and pectin, increases G' and G" and that pectin plays a big role.

5.2.7 Confocal laser scanning microscopy (CLSM)

In food science, it is well known that the structure of foods affects significantly their properties: texture, functionality and appearance (Puri et al., 2017). The microstructure has a main impact on the texture and other physical properties of milk gels (Karam et al., 2016). The confocal laser scanning microscopy (CLSM) was used to observe the microstructure of milk gels, such as dairy desserts (Godoi et al., 2017). Figure 5.5 shows the microstructures of commercial, syrup and mixture dairy desserts (CDD, SDD and MDD). Firstly, for the commercial dairy dessert, it appears homogenous, with relatively small and uniformly dispersed pores, and a network consisting of moderately thin strands. Syrup and mixture dairy desserts, conversely, showed slightly large pores, which are linked with the presence of sugars, and the protein network appeared to be made up from rather thick strands consisting of densely aggregated protein particles.

The milk used for the preparation of syrup and mixture dairy desserts presented protein particles which consist of caseins micelles with associated whey protein. More dense and larger protein aggregates can be predicted between these particles and sugars present in date syrup and powder. This aggregation would cause gelation to occur and this fact might allow for more rearrangement to take place after the gel point, again favoring the formation of more densely aggregated structure.

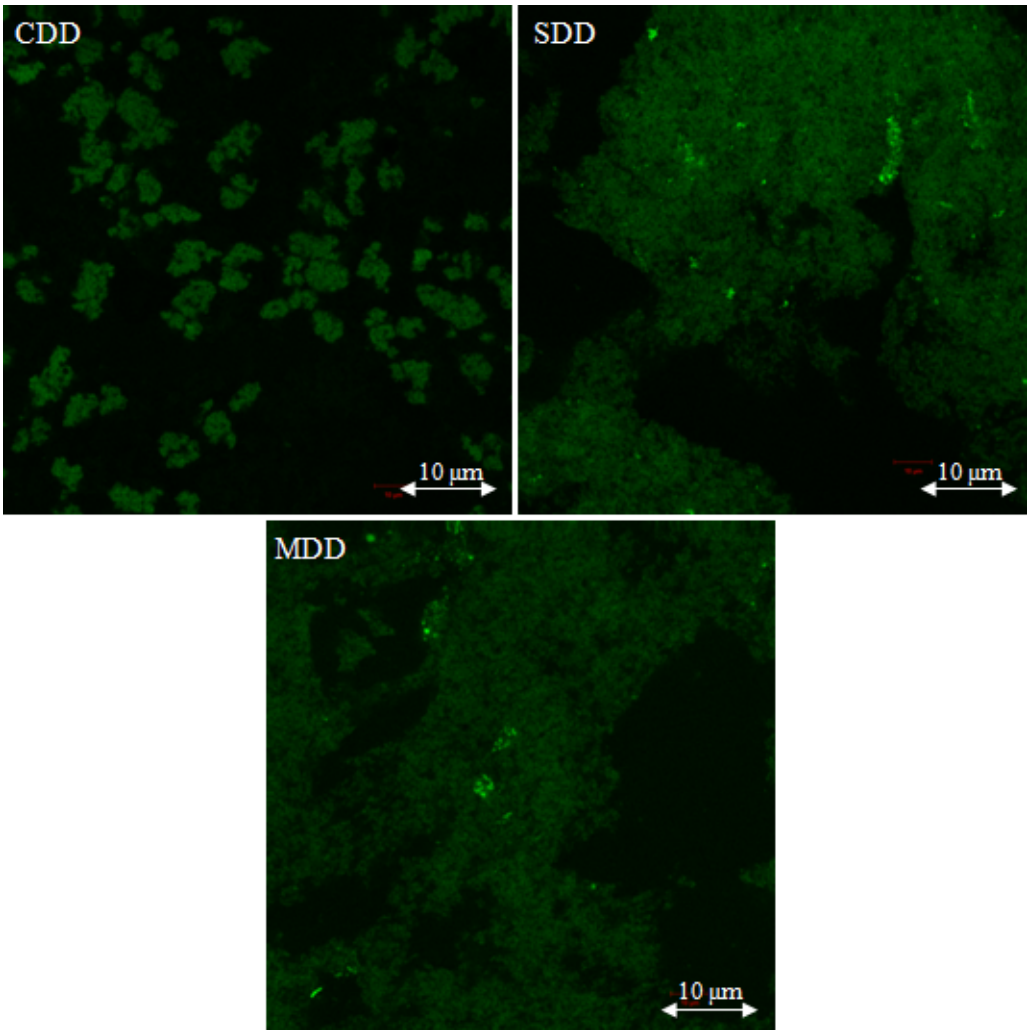


Figure 5.5. Microstructure of dairy desserts obtained by CLSM.

5.3 Conclusion

The application of D-optimal mixture design was effective in optimizing the concentrations of date syrup (14%), date powder (2%) and sucrose (0%) in dairy dessert formulation, based on its sensory evaluation. The physicochemical, phytochemical, microbiological, rheological and microstructural properties of syrup dairy dessert (SDD) and mixture dairy dessert (MDD), studied in this work, were significantly affected by both date syrup and powder additions, compared to the commercial dairy dessert (CDD). This incorporation enhanced the dry matter, lipids, proteins, total phenolic content and antioxidant activity of the formulated dairy desserts, with an absence of protein cross linking and a similarity in the structure of both syrup and mixture dairy desserts. The samples showed a high microbiological quality and a pseudo plastic behaviour. These results lead to conclude that the incorporation of date by-products in dairy desserts to substitute sugar, might be an efficient alternative to formulate new products, with novel characteristics and could be qualified as functional foods with a good acceptability by the consumers.

General Conclusion and Perspectives

General conclusion and perspectives

In conclusion, the reported work highlighted the valorization of the Algerian southern phytogenetic heritage by the valorization of a common date variety, with low commercial value, as a potential innovative sweetner ingredient to substitute sugars, using a simple, fast, energy-efficient, scalable and eco-friendly techniques, to develop a high added value dairy product type "dairy dessert". The formulated dairy desserts, with date syrup and powder, have a sufficiently attractive quality/price ratio.

From the outcome of our investigation it is possible to conclude that Algerian "Degla-Beida" date cultivar was characterized by an important morphological, physicochemical and phytochemical features, providing significant data about the composition of the fruit. The optimized microwave-assisted extraction (MAE) was the best method for extracting date phenolic compounds, compared to ultrasound-assisted and conventional extraction methods (UAE and CE), which leads, not only, to the highest yield (4.27±0.09 mg GAE/g DW), but also reduces considerably the extraction time (2.42 min) at 700 W microwave power. Identifying the optimum extraction parameters for total phenolic compounds from *Phoenix dactylifera* L. would definitely be beneficial in the large-scale industries in terms of saving on the operational cost and time. Using RP-HPLC-PAD-ESI-MS/MS analysis, 44 compounds were identified (sugars, organic acids, nucleosides, carotenoids, proanthocyanidins, lignans, flavonols, flavones, saponin and sterol glucosides) and grouped according to their structural similarity and following their elution order, as well as the Rt, MS data, UV max. and MS^n fragmentation pattern. The antioxidants contained in date fruit could be partly responsible for its ability to reduce oxidative stress. This is in addition to the major role played as a natural supplement which is undeniably much better than chemicals as they could go a long way ahead of pharmaceutical medications in enhancing health and vitality.

The response surface methodology (RSM) was successfully applied to optimize the extraction of total sugar content (TSC) from date fruit, and to compare green technologies (MAE and UAE) with the conventional extraction (CE) method. The second-order polynomial models developed were satisfactory in describing and predicting the sugars extraction. With the application of RSM, the interaction effects among the extraction factors can be accessed as well the solvent usage and extraction time can be reduced. Maximum predicted TSC recoveries under the optimized conditions for MAE, UAE and CE were: 233.25±3.60; 202.90±5.80 and 233.54±5.41 g/L, respectively, which were close to the experimental values: 233.80±1.90; 202.04±3.40 and 234.38±2.43 g/L. HPLC-RID analysis revealed high similarity in the sugar composition of date syrups obtained by MAE (60.11% sucrose, 16.64% glucose and 23.25% fructose) and CE (50.78% sucrose, 20.67% glucose and 28.55% fructose), although a large difference was detected for that obtained by UAE (0.00% sucrose, 46.94% glucose and 53.06% fructose). Microwave-assisted extraction was also the best method for extracting total sugars in terms of extraction yield (233.25±3.60 g/L) and time (1.99 min) at 530 W microwave power. On the other hand, ultrasound-assisted extraction was the one which gives the best yields of reducing sugars, in particular fructose, which is known for its desirable functional properties.

Dairy dessert formulation tests were carried out using the D-Optimal mixture design, to develop the optimal formula based on the sensory analysis. On this basis, an optimal mixture dairy dessert (MDD) formulation with 14% of date syrup, 2% of date powder and 0% of sugar (sucrose) was retained for further analyses, plus syrup and commercial dairy desserts (SDD and CDD). From all the above, it would seem that the formulated dairy desserts, with a total sugar substitution by date syrup and powder, have the best quality. This incorporation enhanced the dry matter, lipids, proteins, total phenolic content, and antioxidant activity of the formulated dairy desserts, with an absence of protein cross-linking and a similarity in the structure of both syrup and mixture dairy desserts. The samples showed a high microbiological quality and a pseudo-plastic behavior. Date syrup and powder may be considered as valuable ingredients for producing food supplements and functional foods. As a result, it can constitute a major asset during the marketing.

Despite all the advantages already mentioned, it is necessary to remain aware of the limitations of the tests carried out at a laboratory scale in batch and on small quantities. The advantages demonstrated at a laboratory scale deserve to be checked at a pilot scale. In order to complete this thesis work, several additional studies should be implemented, in particular:

- To study the impact of other alternative extraction processes on the total sugar yield from the studied date variety;
- To monitor the physicochemical and microbiological properties of the formulated dairy desserts during the storage period (until the expiration date);
- To realase a clinical study (*in vivo*) confirming the functional character of the date fruit, date syrup and dairy desserts;
- To characterize and valorize the residues generated after the preparation of date syrups in the formulation of new functional foods;
- To refine the technico-economic study on the new scheme for valorizing the common dates proposed in this study.

Although this area is widely explored, the research on the preparation of fruit-based products should be continued, especially if the raw material is abundant and at low cost. It is the only way to meet the wishes of the consumer who ask for new products, those of the agricultural producer who is looking for outlets for his fruits, and the wishes of the nutritionist who advises the replacement of artificial products by natural products.

References

1. Abbès, F., Kchaou, W., Blecker, C., Ongena, M., Lognay, G., Attia, H., Besbes, S., 2013. Effect of processing conditions on phenolic compounds and antioxidant properties of date syrup. *Industrial Crops and Products* **44**, 634-642. *https://doi.org/10.1016/j.indcrop.2012.09.008*

2. Abdeldjabbar, M., Messaouda, D., Zhour, R., Cheyma, B., 2020. Comparative Analysis of Total Phenolics, Flavonoid content and Antioxidant profile of date palm (*Phoenix dactylifera* L.) with different watering water from Oued Soufin Algeria. *Asian Journal of Research in Chemistry* **13**(1), 28-32. *DOI:10.5958/09744150*

3. Abdul-Hamid, N.A., Mustaffer, N.H., Maulidiani, M., Mediani, A., Ismail, I.S., Tham, C.L., Shadid, K., Abas, F., 2020. Quality evaluation of the physical properties, phytochemicals, biological activities and proximate analysis of nine Saudi date palm fruit varieties. *Journal of the Saudi Society of Agricultural Sciences* **19**(2), 151-160. *https://doi.org/10.1016/j.jssas.2018.08.004*

4. Abdulrahman, Y.S., Ahmed, F.O., Andrew, G.S., Yusuf, D.O.A., Muhammad, A.B., Eloghosa, O.O.P., Hussaini, A.R., 2020. Nutritional Properties of Some Selected Date Palm (*Phoenix dactylifera* L) Land Races in Nigeria. *World Journal of Food Science and Technology* **4**(1), 1-7. *DOI:10.11648/j.wjfst.20200401.11*

5. Abid, W., Mahmoud, I.B., Masmoudi, S., Triki, M.A., Mounier, S., Ammar, E., 2020. Physico-chemical and spectroscopic quality assessment of compost from date palm (*Phoenix dactylifera* L.) waste valorization. *Journal of Environmental Management* **264**, 110492. *https://doi.org/10.1016/j.jenvman.2020.110492*

6. Abou-Taleb, K.A., Galal, G.F., 2018. A comparative study between one-factor-at-a-time and minimum runs resolution-IV methods for enhancing the production of polysaccharide by *Stenotrophomonas daejeonensis* and *Pseudomonas geniculate*. *Annals of Agricultural Sciences* **63**(2), 173-180. *https://doi.org/10.1016/j.aoas.2018.11.002*

7. Abu-Reidah, I.M., Gil-Izquierdo, Á., Medina, S., Ferreres, F., 2017. Phenolic composition profiling of different edible parts and by-products of date palm (*Phoenix dactylifera* L.) by using HPLC-DAD-ESI/MS^n. *Food research international* **100**(3), 494-500. *https://doi.org/10.1016/j.foodres.2016.10.018*

8. Adeosun, A.M., Oni, S.O., Ighodaro, O.M., Durosinlorun, O.H., Oyedele, O.M., 2016. Phytochemical, minerals and free radical scavenging profiles of *Phoenix dactilyfera* L. seed extract. *Journal of Taibah University Medical Sciences* **11**(1), 1-6. *https://doi.org/10.1016/j.jtumed.2015.11.006*

9. Agboola, O.S., Adejumo, A.L., 2013. Nutritional composition of the fruit of the Nigerian wild date palm, *Phoenix dactylifera. World Journal of Dairy and Food Sciences* **8**(2), 196-200. *DOI:10.5829/idosi.wjdfs.2013.8.2.81178*

10. Ahmed, J., Al-Jasass, F.M., Siddiq, M., 2014. Date fruit composition and nutrition. *Dates: Postharvest Science, Processing Technology and Health Benefits, John Wiley and Sons, Ltd*, 261-283.

11. Ahmed, H., Nawel, O., Ghalem, R. B., Zouaoui, B., Djilali, B., 2016. Effect of Total or Partial Substitution of Cacao and Sucrose by Date Powders Variety H'lowa on the Some Quality of Dairy Creamed Dessert. *Journal of Applied, Environemental and Biological Sciences* **6**(7), 100-108.

12. Akonor, P.T., 2020. Optimization of a fruit juice cocktail containing soursop, pineapple, orange and mango using mixture design. *Scientific African* **8**, e00368. *https://doi.org/10.1016/j.sciaf.2020.e00368*

13. Al-Khayri, J., Jain, S., Johnson, D., 2015. Date palm genetic resources and utilization, Volume 1: *Africa and the Americas. DOI:10.1007/978-94-017-97078*

14. Al-Turki, S., Shahba, M.A., Stushnoff, C., 2010. Diversity of antioxidant properties and phenolic content of date palm (*Phoenix dactylifera* L.) fruits as affected by cultivar and location. *Journal of Food Agriculture and Environement* **8** (1), 253-260.

15. Al Alawi, R., Alhamdani, M.S.S., Hoheisel, J.D., Baqi, Y., 2020. Antifibrotic and tumor microenvironment modulating effect of date palm fruit (*Phoenix dactylifera* L.) extracts in pancreatic cancer. *Biomedicine and Pharmacotherapy* **121**, 109522. *https://doi.org/10.1016/j.biopha.2019.109522*

16. Al-Farsi, M.A., Lee, C.Y., 2008. Nutritional and functional properties of dates: a review. *Critical reviews in food science and nutrition* **48**, 877-887. *https://doi.org/10.1080/10408390701724264*

17. Al Harthi, S., Mavazhe, A., Al Mahroqi, H., Khan, S., 2015. Quantification of phenolic compounds, evaluation of physicochemical properties and antioxidant activity of four date (*Phoenix dactylifera* L.) varieties of Oman. *Journal of Taibah University Medical Sciences* **10**(3), 346-352. *https://doi.org/10.1016/j.jtumed.2014.12.006*

18. Al Juhaimi, F., Özcan, M.M., Uslu, N., Ghafoor, K., Babiker, E.E., Mohamed Ahmed, I.A., 2020. Bioactive properties, fatty acid compositions, and phenolic

compounds of some date palm (*Phoenix dactylifera* L.) cultivars. *Journal of Food Processing and Preservation* **44**(5), e14432. https://doi.org/10.1111/jfpp.14432

19. Alam, M.Z., Alhebsi, M.S., Ghnimi, S., Kamal-Eldin, A., 2021. Inability of total antioxidant activity assays to accurately assess the phenolic compounds of date palm fruit (*Phoenix dactylifera* L.). *NFS journal* 22, 32-40. *https://doi.org/10.1016/j.nfs.2021.01.001*

20. Alem, C., Ennassir, J., Benlyas, M., Mbark, A.N., Zegzouti, Y.F., 2017. Phytochemical compositions and antioxidant capacity of three date (*Phoenix dactylifera* L.) seeds varieties grown in the South East Morocco. *Journal of the Saudi Society of Agricultural Sciences* **16**(4), 350-357. https://doi.org/10.1016/j.jssas.2015.11.002

21. Aljaloud, S., Colleran, H.L., Ibrahim, S.A., 2020. Nutritional Value of Date Fruits and Potential Use in Nutritional Bars for Athletes. *Food and Nutrition Sciences* **11**(6), 463.

22. Alshehri, S.M., Al-Lohedan, H.A., Al-Farraj, E., Alhokbany, N., Chaudhary, A.A., Ahamad, T., 2016. Macroporous natural capsules extracted from *Phoenix dactylifera* L. spore and their application in oral drugs delivery. *International journal of pharmaceutics* **504**(1), 39-47. *https://doi.org/10.1016/j.ijpharm.2016.02.049*

23. Alshwyeh, H.A., 2020. Phenolic profiling and antibacterial potential of Saudi Arabian native date palm (*Phoenix dactylifera*) cultivars. *International Journal of Food Properties* **23**(1), 627-638. https://doi.org/10.1080/10942912.2020.1751196

24. Amaral-Fonseca, M., Morellon-Sterling, R., Fernández-Lafuente, R., Tardioli, P.W., 2021. Optimization of simultaneous saccharification and isomerization of dextrin to high fructose syrup using a mixture of immobilized amyloglucosidase and glucose isomerase. *Catalysis Today* **362**, 175-183. *https://doi.org/10.1016/j.cattod.2020.03.021*

25. Amellal, H., 2008. Aptitudes technologique de quelques variétés communes de dattes: formulation d'un yaourt naturellement sucré et aromatisé. Thèse de doctorat, Université de Boumerdès-M'hamed Bougara, Algérie, 131.

26. Amerinasab, A., Labbafi, M., Mousavi, M., Khodaiyan, F., 2015. Development of a novel yoghurt based on date liquid sugar: physicochemical and sensory characterization. *Journal of food science and technology* **52**, 6583-6590. *https://doi.org/10.1007/s13197-015-1716-4*

27. Amin, W.F., 2018. Occurrence of Bacillus cereus in some milk-based desserts. *Assiut Veterinary Medical Journal* **64**, 41-46.

28. Amiour, S.D., Hambaba, L., 2016. Effect of pH, temperature and some chemicals on polyphenoloxidase and peroxidase activities in harvested Deglet Nour and Ghars dates. *Postharvest Biology and Technology* **111**, 77-82. *https://doi.org/10.1016/j.postharvbio.2015.07.027*

29. Ammar, I., Gharsallah, H., Brahim, A.B., Attia, H., Ayadi, M., Hadrich, B., Felfoul, I., 2020. Optimization of gluten-free sponge cake fortified with whey protein concentrate using mixture design methodology. *Food chemistry* **343**, 128457.

30. Amorós, A., Rivera, D., Larrosa, E., Obón, C., 2014. Physico-chemical and functional characteristics of date fruits from different Phoenix species (Arecaceae). *Fruits* **69**(4), 315-323. *DOI:10.1051/fruits/2014020*

31. Arfaoui, L., 2020. Total polyphenol content and radical scavenging activity of functional yogurt enriched with dates. *Czech Journal of Food Sciences* **38**(5), 287–292. *https://doi.org/10.17221/28/2020-CJFS*

32. Asma, L., Ahmed, M., Khadidja, M., 2020. Microbiological and Physicochemical attributes of two date Cultivars Hmira and Feggous' in Abadela, Bechar Oasis, South-West of Algeria. *South Asian Journal of Experimental Biology* **9**(5), 207-213.

33. Awad, S., Zhou, Y., Katsou, E., Li, Y., Fan, M., 2020. A Critical Review on Date Palm Tree (Phoenix dactylifera L.) Fibres and Their Uses in Bio-composites. *Waste and Biomass Valorization*, 1-35. *https://doi.org/10.1007/s12649-020-011-05-2*

34. Awan, K.A., Butt, M.S., Sharif, M.K., Hussain, F., 2018. Compositional profiling of selected Pakistani date cultivars. *Pakistan Journal of Agricultural Sciences* **55**(3), 575-581. *DOI:10.21162/PAKJAS/18.6694*

35. Azaïs-Braesco, V., Sluik, D., Maillot, M., Kok, F., Moreno, L.A., 2017. A review of total and added sugar intakes and dietary sources in Europe. *Nutrition Journal* **16**, 6. *https://doi.org/10.1186/s12937-016-0225-2*

36. Bagherzadeh Karimi, A., Elmi, A., Mirghafourvand, M., Baghervand Navid, R., 2020. Effects of date fruit (*Phoenix dactylifera* L.) on labor and delivery outcomes: a systematic review and meta-analysis. *BMC Pregnancy and Childbirth* **20**, 1-14. *https://doi.org/10.1186/s12884-020-02915-x*

37. Bamawa, C., Ndjele, L., Foma, F., 2016. Characterization of leaf phenolic compounds of *Sabicea johnstonii* by HPLC-MS^n. *Journal of Natural Products and Resources* **2**(2), 86–89.

38. Bedjaoui, H., Benbouza, H., 2020. Assessment of phenotypic diversity of local Algerian date palm (*Phoenix dactylifera* L.) cultivars. *Journal of the Saudi Society of Agricultural Sciences* **19**(1), 65-75. *https://doi.org/10.1016/j.jssas.2018.06.002*

39. Beg, S., 2021. Mixture Designs and Their Applications in Pharmaceutical Product Development, Design of Experiments for Pharmaceutical Product Development. *Springer*, 87-96. *https://doi.org/10.1007/978-981-33-4717-5-8*

40. Benidir, M., El Massoudi, S., El Ghadraoui, L., Lazraq, A., Benjelloun, M., Errachidi, F., 2020. Study of Nutritional and Organoleptic Quality of Formulated Juices from Jujube (*Ziziphus lotus* L.) and Dates (*Phoenix dactylifera* L.) Fruits. *The Scientific World Journal* **2020**, 1-9. *https://doi.org/10.1155/2020/9872185*

41. Benmeziane-Derradji, F., 2019. Nutritional value, phytochemical composition, and biological activities of Middle Eastern and North African date fruit: an overview. *Euro-Mediterranean Journal for Environmental Integration* **4**, 39. *https://doi.org/10.1007/s41207-019-0132-y*

42. Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta* **76** (5), 965-977. *https://doi.org/10.1016/j.talanta.2008.05.019*

43. Bezerra, M.A., Lemos, V.A., Novaes, C.G., de Jesus, R.M., Souza Filho, H.R., Araújo, S.A., Alves, J.P.S., 2020. Application of mixture design in analytical chemistry. *Microchemical Journal* **152**, 104336. *https://doi.org/10.1016/j.microc.2019.104336*

44. Bianchin, M., de Lima, H.H.C., Monteiro, A.M., de Toledo Benassi, M., 2020. Optimization of ultrasonic-assisted extraction of kahweol and cafestol from roasted coffee using response surface methodology. *LWT* **117**, 108593. *https://doi.org/10.1016/j.lwt.2019.108593*

45. Bibi Sadeer, N., Montesano, D., Albrizio, S., Zengin, G., Mahomoodally, M.F., 2020. The versatility of antioxidant assays in food science and safety—Chemistry, applications, strengths, and limitations. *Antioxidants* **9**, 709. *DOI:10.3390/antiox9080709*

46. Bimakr, M., Rahman, R.A., Ganjloo, A., Taip, F.S., Salleh, L.M., Sarker, M.Z.I., 2012. Optimization of supercritical carbon dioxide extraction of bioactive flavonoid

compounds from spearmint (*Mentha spicata* L.) leaves by using response surface methodology. *Food and Bioprocess Technology* **5**, 912-920. *https://doi.org/10.1007/s11947-010-0504-4*

47. Botinestean, C., Hossain, M., Mullen, A.M., Auty, M.A., Kerry, J.P., Hamill, R.M., 2020. Optimization of textural and technological parameters using response surface methodology for the development of beef products for older consumers. *Journal of texture studies* **51**, 263-275. *DOI:10.1111/jtxs.12467*

48. Bouguedoura, N., Bennaceur, M., Babahani, S., Benziouche, S.E., 2015. Date palm status and perspective in Algeria, Date palm genetic resources and utilization. *Springer*, 125-168. *DOI:10.1007/978-94-017-9694-1-4*

49. Boukhiar, A., 2009. Analyse du processus traditionnel d'obtention du vinaigre de dattes tel qu'appliqué au sud Algérie : essai d'optimisation. Mémoire de magistère, centre universitaire de Boumerdes.

50. Boulekbache-Makhlouf, L., Slimani, S., Madani, K., 2013. Total phenolic content, antioxidant and antibacterial activities of fruits of *Eucalyptus globulus* cultivated in Algeria. *Industrial Crops and Products* **41**, 85-89. *https://doi.org/10.1016/j.indcrop.2012.04.019*

51. Cadena, R., Cruz, A., Faria, J., Bolini, H., 2012. Reduced fat and sugar vanilla ice creams: Sensory profiling and external preference mapping. *Journal of dairy science* **95**, 4842-4850. *https://doi.org/10.3168/jds.2012-5526*

52. Câmara, J.S., Albuquerque, B.R., Aguiar, J., Corrêa, R.C., Gonçalves, J.L., Granato, D., Pereira, J.A., Barros, L., Ferreira, I.C., 2021. Food Bioactive Compounds and Emerging Techniques for Their Extraction: Polyphenols as a Case Study. *Foods* **10**, 37. *https://dx.doi.org/10.3390/foods10010037*

53. Carbone, K., Amoriello, T., Iadecola, R., 2020. Exploitation of Kiwi Juice Pomace for the Recovery of Natural Antioxidants through Microwave-Assisted Extraction. *Agriculture* **10**, 435. *DOI:10.3390/agriculture10100435*

54. Chaira, N., Smaali, I., Besbes, S., Mrabet, A., Lachiheb, B., Ferchichi, A., 2011. Production of fructose rich syrups using invertase from date palm fruits. *Journal of Food Biochemistry* **35**, 1576-1582. *DOI:10.1111/j.1745-4514.2010.00487.x*

55. Chan, C.H., Yusoff, R., Ngoh, G.C., 2014. Optimization of microwave-assisted extraction based on absorbed microwave power and energy. *Chemical Engineering Science* **111**, 41-47. *https://doi.org/10.1016/j.ces.2014.02.011*

56. Chemat, F., Vian, M.A., Fabiano-Tixier, A.S., Nutrizio, M., Jambrak, A.R., Munekata, P.E., Lorenzo, J.M., Barba, F.J., Binello, A., Cravotto, G., 2020. A review of sustainable and intensified techniques for extraction of food and natural products. *Green Chemistry* **22**, 2325-2353. *DOI:10.1039/C9GC03878G*

57. Chouhan, P., Saini, T., 2016. D-optimal design and development of microemulsion based transungual drug delivery formulation of ciclopirox olamine for treatment of onychomycosis. *Indian Journal of Pharmaceutical Sciences* **78**, 498-511.

58. da Silva, S.C., Fernandes, I.P., Barros, L., Fernandes, Â., Alves, M.J., Calhelha, R.C., Pereira, C., Barreira, J.C., Manrique, Y., Colla, E., 2019. Spray-dried Spirulina platensis as an effective ingredient to improve yogurt formulations: Testing different encapsulating solutions. *Journal of Functional Foods* **60**, 103427. *https://doi.org/10.1016/j.jff.2019.103427*

59. da Rocha, C.B., Noreña, C.P.Z., 2020. Microwave-Assisted Extraction and Ultrasound-Assisted Extraction of Bioactive Compounds from Grape Pomace. *International Journal of Food Engineering* **16**. *https://doi.org/10.1515/ijfe-20190191*

60. Dable-Tupas, G., Otero, M.C.B., Bernolo, L., 2020. Functional Foods and Health Benefits, Functional Foods and Nutraceuticals. *Springer*, 1-11.

61. David, J., Mahanty, B., 2021. Optimized ciprofloxacin release from citric acid crosslinked starch-PVA hydrogel film: modelling with mixture design. *Journal of Polymer Research* **28**, 1-11. *https://doi.org/10.1007/s10965-020-02397-7*

62. de Bertoldi, C., De Leo, M., Ercoli, L., Braca, A., 2012. Chemical profile of Festuca arundinacea extract showing allelochemical activity. *Chemoecology* **22**, 13-21. *https://doi.org/10.1007/s00049-011-0092-4*

63. de Rosso, V.V., Mercadante, A.Z., 2007. Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. *Journal of Agricultural and Food Chemistry* **55**, 5062-5072. *https://doi.org/10.1021/jf0705421*

64. de Sousa, M.C., Dos Santos, W.M., da Silva, J.M.O., Ramos, F.P., de Freitas, A.S., Neta, M.C.A., Dos Santos, K.M.O., Buriti, F.C.A., Florentino, E.R., 2021. Non-fermented Dairy Desserts with Potentially Probiotic *Autochthonous Lactobacilli* and Products from Peel of Jabuticaba (*Myrciaria cauliflora*). *Probiotics and Antimicrobial Proteins*, 1-11. *https://doi.org/10.1007/s12602-020-09731-x*

65. Dean, A., Voss, D., Draguljić, D., 2017. Response surface methodology, Design and analysis of experiments. *Springer*, 565-614.

66. Derouich, M., Meziani, R., Bourkhis, B., Filali-Zegzouti, Y., Alem, C., 2020. Nutritional, mineral and organic acid composition of syrups produced from six Moroccan date fruit (*Phoenix dactylifera* L.) varieties. *Journal of Food Composition and Analysis* **93**, 103591. *https://doi.org/10.1016/j.jfca.2020.103591*

67. Deshpande, N.M., Deshpande, M.M., 2017. Date fruit (*Phoeinix Dactylifera* Linn)–A review on nutritional values, phytochemicals and pharmacological actions. *World Journal of Pharmaceutical Research*, **6**(8), 419-426. *DOI:10.20959/wjpr20178-8943*

68. Dhaouadi, K., Raboudi, F., Estevan, C., Barrajon, E., Vilanova, E., Hamdaoui, M., Fattouch, S., 2011. Cell viability effects and antioxidant and antimicrobial activities of Tunisian date syrup (Rub El Tamer) polyphenolic extracts. *Journal of agricultural and food chemistry* **59**(1), 402-406. *https://doi.org/10.1021/jf103388m*

69. Di Cagno, R., Filannino, P., Cavoski, I., Lanera, A., Mamdouh, B.M., Gobbetti, M., 2017. Bioprocessing technology to exploit organic palm date (*Phoenix dactylifera* L. cultivar Siwi) fruit as a functional dietary supplement. *Journal of Functional Foods* **31**, 9-19. *https://doi.org/10.1016/j.jff.2017.01.033*

70. Djaoud, K., Arkoub-Djermoune, L., Remini, H., Sait, S., Tazarourte, M., Hadjal, S., Romero, A., Madani, K., Boulekbache-Makhlouf, L., 2019. Syrup from common date variety (*Phoenix dactylifera* L.): Optimization of sugars extraction and their quantification by high performance liquid chromatography. *Current Nutrition and Food Science* **15**, 1-13. *DOI:10.2174/1573401315666190115160950*

71. Djaoud, K., Boulekbache-Makhlouf, L., Yahia, M., Mansouri, H., Mansouri, N., Madani, K., Romero, A., 2020a. Dairy dessert processing: Effect of sugar substitution by date syrup and powder on its quality characteristics. *Journal of Food Processing and Preservation* **44**, e14414. https://doi.org/10.1111/jfpp.14414

72. Djaoud, K., Daglia, M., Sokeng, A.J., Kermiche, F., Arkoub, L., Madani, K., Makhlouf, L.B., 2020b. RP-HPLC-PDA-ESI-MS/MS screening of bioactive compounds from Degla-Beida dates: Conventional and green extraction technologies. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology* **44**(1), 58-81. *https://doi.org/10.35219/foodtechnology.2020.1.04*

73. Djaoudene, O., Bey, M.B., Louaileche, H., 2019. Physicochemical Characteristics and Nutritional Compositions of Some Date (*Phoenix dactylifera* L.) Fruit Cultivars. Acta Universitatis Cibiniensis. *Series E: Food Technology* **23**, 129-138.

74. Djouab, A., Benamara, S., Gougam, H., Amellal, H., Hidous, K., 2016. Physical and antioxidant properties of two Algerian date fruit species (*Phoenix dactylifera*

L. and *Phoenix canariensis* L.). *Emirates Journal of Food and Agriculture*, 601-608.

75. Doma, M.B., EL-Shahawy, S.M.M., Grawish, S.A.H., 2013. Processing of date honey. *Journal of Food and Dairy Sciences* **4**, 51-64.

76. Dorneles, M.S., Noreña, C.P.Z., 2020. Microwave-assisted extraction of bioactive compounds from Araucaria angustifolia bracts followed by encapsulation. *Journal of Food Processing and Preservation* **44**(6), e14484. *https://doi.org/10.1111/jfpp.14484*

77. Durward, C., 2020. Added Sugars: What You Need to Know. *Food and nutrition*, 1-5. *https://digitalcommons.usu.edu/extension-curall/2115*

78. Ehirim, F., Onyeneke, E., 2013. Physico-chemical and organoleptic properties of yoghurt manufactured with cow milk and goat milk. *Academic Research International* **4**(4), 245-252.

79. El-Mously, H., 2019. Rediscovering Date Palm by-products: an Opportunity for Sustainable Development. *By-Products of Palm Trees and Their Applications* **11**, 3-61. *https://doi.org/10.21741/9781644900178-1*

80. El-Mously, H., Midani, M., Wagih, M., 2019. By-products of palm trees and their applications. 1st World Conference on By-Products of Palm Trees and Their Applications (ByPalma), Aswan, Egypt. *Materials Research Forum LLC* **11**. *https://doi.org/10.21741/9781644900178-4*

81. El-Naggar, M.E., Shaarawy, S., Hebeish, A., 2018. Multifunctional properties of cotton fabrics coated with in situ synthesis of zinc oxide nanoparticles capped with date seed extract. *Carbohydrate Polymers* **181**, 307-316. *https://doi.org/10.1016/j.carbpol.2017.10.074*

82. El-Sohaimy, S., Hafez, E., 2010. Biochemical and nutritional characterizations of date palm fruits (*Phoenix dactylifera* L.). *Journal of Applied Sciences Research* **6**(8), 1060-1067.

83. El Sohaimy, S., Abdelwahab, A., Brennan, C.S., Aboul-Enein, A., 2015. Phenolic content, antioxidant and antimicrobial activities of Egyptian date palm (*Phoenix dactylifera L.*) fruits. *Australian Journal of Basic and Applied Sciences* **9**(1), 141-147.

84. Erickson, S., Carr, J., 2020. The technological challenges of reducing the sugar content of foods. *Nutrition Bulletin* **45**, 309-314. *DOI:10.1111/nbu.12454*

85. FAOSTAT, 2020. *Food and agriculture organization of the United Nations*, from *http:faost at.fao.org/site/339/default.aspx*

86. Floss, D.S., Schliemann, W., Schmidt, J., Strack, D., Walter, M.H., 2008. RNA interference-mediated repression of MtCCD1 in mycorrhizal roots of Medicago truncatula causes accumulation of C27 apocarotenoids, shedding light on the functional role of CCD1. *Plant physiology* **148**, 1267-1282. *www.plantphysiol.org/cgi/doi/10.1104/pp.108.125062*

87. Gad, A., Kholif, A., Sayed, A., 2010. Evaluation of the nutritional value of functional yogurt resulting from combination of date palm syrup and skim milk. *American Journal of Food Technology* **5** (4), 250-259.

88. Ganbi, H., 2012. Production of nutritious high quality date (Phoenix dactylifera) fruits syrup (Dibs) by using some novel technological approaches. *Journal of Applied Sciences Research*, 1524-1538. *http://www.aensiweb.com/jasr/jasr/20133012564*

89. Gantait, S., El-Dawayati, M.M., Panigrahi, J., Labrooy, C., Verma, S.K., 2018. The retrospect and prospect of the applications of biotechnology in *Phoenix dactylifera* L. *Applied microbiology and biotechnology* **102**, 8229-8259. *https://doi.org/10.1007/s00253-018-9232-x*

90. Garcia-Vaquero, M., Ummat, V., Tiwari, B., Rajauria, G., 2020. Exploring Ultrasound, Microwave and Ultrasound–Microwave Assisted Extraction Technologies to Increase the Extraction of Bioactive Compounds and Antioxidants from Brown Macroalgae. *Marine Drugs* **18**, 172. *DOI:10.3390/md18030172*

91. Georgé, S., Brat, P., Alter, P., Amiot, M.J., 2005. Rapid determination of polyphenols and vitamin C in plant-derived products. *Journal of Agricultural and Food Chemistry* **53**, 1370-1373.

92. Gheisari, H., Heydari, S., Basiri, S., 2020. The effect of date versus sugar on sensory, physicochemical, and antioxidant properties of ice cream. *Iranian Journal of Veterinary Research* **21**(1), 9-14.

93. Ghnimi, S., Umer, S., Karim, A., Kamal-Eldin, A., 2017. Date fruit (*Phoenix dactylifera* L.): An underutilized food seeking industrial valorization. *NFS journal* **6**, 1-10. *https://doi.org/10.1016/j.nfs.2016.12.001*

94. Ghorbannezhad, P., Bay, A., Yolmeh, M., Yadollahi, R., Moghadam, J.Y., 2016. Optimization of coagulation–flocculation process for medium density fiberboard (MDF) wastewater through response surface methodology. *Desalination and*

Water Treatment **57**, 26916-26931. *http://dx.doi.org/10.1080/19443994.2016.1170636*

95. Godoi, F.C., Bhandari, B.R., Prakash, S., 2017. Tribo-rheology and sensory analysis of a dairy semi-solid. *Food Hydrocolloids* **70**, 240-250. *https://doi.org/10.1016/j.foodhyd.2017.04.011*

96. González, M., Barrios, S., Budelli, E., Pérez, N., Lema, P., Heinzen, H., 2020. Ultrasound assisted extraction of bioactive compounds in fresh and freeze-dried *Vitis vinifera* cv Tannat grape pomace. *Food and Bioproducts Processing.* **124**, 378-386. *https://doi.org/10.1016/j.fbp.2020.09.012*

97. Gourchala, F., Ouazouaz, M., Mihoub, F., Henchiri, C., 2015. Compositional analysis and sensory profile of five date varieties grown in south Algeria. *Journal of Chemical and Pharmaceutical Research* **7**(2), 511-518.

98. Gros Balthazard, M., Battesti, V., Ivorra, S., Paradis, L., Aberlenc, F., Zango, O., Zehdi Azouzi, S., Moussouni, S., Naqvi, S.A., Newton, C., 2020. On the necessity of combining ethnobotany and genetics to assess agrobiodiversity and its evolution in crops: A case study on date palms (*Phoenix dactylifera* L.) in Siwa Oasis, Egypt. *Evolutionary Applications.* **13**, 1818–1840. *DOI:10.1111/eva.12930*

99. Gurditta, H., Patel, A.A., Arora, S., 2019. Selection of dietary fibres for sucrose replacement in functional Chhana murki (Indian Cottage cheese based dessert) and their effect on sensory, physical and instrumental texture parameters. *International journal of dairy technology* **72**, 633-642. *DOI:10.1111/1471-0307.12624*

100. Haida, Z., Hakiman, M., 2019. A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. *Food science and nutrition* **7**, 1555-1563. *DOI:10.1002/fsn3.1012*

101. Hamad, I., AbdElgawad, H., Al Jaouni, S., Zinta, G., Asard, H., Hassan, S., Hegab, M., Hagagy, N., Selim, S., 2015. Metabolic analysis of various date palm fruit (*Phoenix dactylifera* L.) cultivars from Saudi Arabia to assess their nutritional quality. *Molecules* **20**, 13620-13641. *DOI:10.3390/molecules200813620*

102. Hamza-Chaffai, A., 1990. Caractérisation rhéologique d'un lait gélifié chocolaté: optimisation de la technologie de fabrication. *Le Lait* **70**, 155-167.

103. Han, D., Zhu, T., Row, K.H., 2011. Ultrasonic extraction of phenolic compounds from Laminaria japonica Aresch using ionic liquid as extraction solvent. Bull. *Korean Chem. Soc* **32**(7), 2212-2216. *DOI:10.5012/bkcs.2011.32.7.2212*

104. Handa, C.L., de Lima, F.S., Guelfi, M.F.G., Georgetti, S.R., Ida, E.I., 2016. Multi-response optimisation of the extraction solvent system for phenolics and antioxidant activities from fermented soy flour using a simplex-centroid design. *Food chemistry* **197**, 175-184. *https://doi.org/10.1016/j.foodchem.2015.10.124*

105. Hanson, R., Phillips, J., 1981. Chemical composition. Manual of methods for general bacteriology. *American Society for Microbiology, Washington, DC*, 328-364.

106. Hanuš, O., Samková, E., Křížová, L., Hasoňová, L., Kala, R., 2018. Role of fatty acids in milk fat and the influence of selected factors on their variability-A review. *Molecules* **23**, 1636. *DOI:10.3390/molecules23071636*

107. Hinkaew, J., Sahasakul, Y., Tangsuphoom, N., Suttisansanee, U., 2020. The Effect of Cultivar Variation on Total Phenolic Contents and Antioxidant Activities of Date Palm Fruit (*Phoenix Dactylifera* L.). *Current Research in Nutrition and Food Science Journal* **8**, 155-163. *http://dx.doi.org/10.12944/CRNFSJ.8.1.14*

108. Hussain Mallhi, T., Qadir, M.I., Ali, M., Ahmad, B., Khan, Y.H., 2014. Ajwa Date (*Phoenix dactylifera*): An Emerging Plant in Pharmacological Research. *Pakistan journal of pharmaceutical sciences* **27**(3), 607-616.

109. Hussain, M.I., Farooq, M., Syed, Q.A., 2020. Nutritional and biological characteristics of the date palm fruit (*Phoenix dactylifera* L.)–A review. *Food Bioscience* **34**, 100509. *https://doi.org/10.1016/j.fbio.2019.100509*

110. Idowu, A.T., Igiehon, O.O., Adekoya, A.E., Idowu, S., 2020. Dates palm fruits: A review of their nutritional components, bioactivities and functional food applications. *AIMS Agriculture and Food* **5**, 734.

111. Iqbal, S., Ayyub, A., Iqbal, H., Chen, X.D., 2021. Protein microspheres as structuring agents in lipids: potential for reduction of total and saturated fat in food products. *Journal of the Science of Food and Agriculture* **101**, 820-830. *DOI:10.1002/jsfa.10645*

112. Ivanova, M., Petkova, N., Balabanova, T., Ognyanov, M., Vlaseva, R., 2018. Food design of dairy desserts with encapsulated cornelian cherry, chokeberry and blackberry juices. *The Annals of the University of Dunarea de Jos of Galati. Fascicle VI. Food Technology* **42** (1), 137-146.

113. Jahromi, M., Niakousari, M., 2018. Development and characterisation of a sugar free milk based dessert formulation with fig (*Ficus carica* L.) and carboxymethylcellulose. *International journal of dairy technology* **71**, 801-809. *DOI:10.1111/1471-0307.12484*

114. Jasni, A.B., Kamyab, H., Chelliapan, S., Arumugam, N., Krishnan, S., Din, M.F.M., 2020. Treatment of Wastewater Using Response Surface Methodology: A Brief Review. *Chemical Engineering Transactions* **78**, 535-540. *https://doi.org/10.3303/CET2078090*

115. JORA: Journal officiel de la république Algérienne N° 39, 2017. Critères microbiologiques applicables aux denrées alimentaires: Laits et produits laitiers.

116. Jridi, M., Souissi, N., Salem, M.B., Ayadi, M., Nasri, M., Azabou, S., 2015. Tunisian date (*Phoenix dactylifera* L.) by-products: Characterization and potential effects on sensory, textural and antioxidant properties of dairy desserts. *Food chemistry* **188**, 8-15. *https://doi.org/10.1016/j.foodchem.2015.04.107*

117. Kamairudin, N., Hoong, S.S., Abdullah, L.C., Ariffin, H., Biak, D.R.A., 2021. Optimisation of Epoxide Ring-Opening Reaction for the Synthesis of Bio-Polyol from Palm Oil Derivative Using Response Surface Methodology. *Molecules* **26**, 648. *https://doi.org/ 10.3390/molecules26030648*

118. Karam, M.C., Gaiani, C., Hosri, C., Hussain, R., Scher, J., 2016. Textural properties of acid milk gels: Effects of dairy protein powder rehydration state. *Journal of Food Processing and Preservation* **40**, 414-421. *DOI:10.1111/jfpp.12618*

119. Kaur, L., Lamsar, H., López, I.F., Filippi, M., Ong Shu Min, D., Ah-Sing, K., Singh, J., 2021. Physico-Chemical Characteristics and *In Vitro* Gastro-Small Intestinal Digestion of New Zealand Ryegrass Proteins. *Foods* **10**, 331. *https://doi.org/10.3390/foods10020331*

120. Kchaou, W., Abbès, F., Blecker, C., Attia, H., Besbes, S., 2013. Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties (*Phoenix dactylifera* L.). *Industrial Crops and Products* **45**, 262-269. *https://doi.org/10.1016/j.indcrop.2012.12.028*

121. Kchaou, W., Abbès, F., Mansour, R.B., Blecker, C., Attia, H., Besbes, S., 2016. Phenolic profile, antibacterial and cytotoxic properties of second grade date extract from Tunisian cultivars (*Phoenix dactylifera* L.). *Food chemistry* **194**, 1048-1055. https://doi.org/10.1016/j.foodchem.2015.08.120

122. Kermiche, F., Boulekbache–Makhlouf, L., Félix, M., Harkat-Madouri, L., Remini, H., Madani, K., Romero, A., 2018. Effects of the incorporation of cantaloupe pulp in yogurt: Physicochemical, phytochemical and rheological properties. *Food Science and Technology International* **24**, 585-597. *DOI: 10.1177/1082013218776701*

123. Keshtkaran, M., Mohammadifar, M.A., Asadi, G.H., Nejad, R.A., Balaghi, S., 2013. Effect of gum tragacanth on rheological and physical properties of a flavored milk drink made with date syrup. *Journal of dairy science* **96** (8), 4794-4803. *https://doi.org/10.3168/jds.2012-5942*

124. Khan, A.L., Asaf, S., Lee, I.J., Al-Harrasi, A., Al-Rawahi, A., 2018. First chloroplast genomics study of *Phoenix dactylifera* (var. Naghal and Khanezi): A comparative analysis. *PloS one* **13**, e0200104. *https://doi.org/10.1371/journal.pone.0200104*

125. Khapre, A., Shah, U.A., 2016. Standardization of technology for development of Soft date (*Pheonix dactylifera*) toffee as a nutritional enrichment of confectionery. *Asian Journal of Dairy and Food Research* **35**(4), 335-337.

126. Kilic, D., Ebegil, M., Bayrak, H., Ozkaya, B., Avsar, B.A., 2019. Optimization Of Multi Responses Using Data Envelopment Analysis: The Application in Food Industry. *Gazi University Journal of Science* **32**(3), 1083-1090. *DOI:10.35378/gujs.394984*

127. Köksoy, A., Kılıç, M., 2003. Effects of water and salt level on rheological properties of ayran, a Turkish yoghurt drink. *International Dairy Journal* **13**(10), 835-839. *https://doi.org/10.1016/S0958-6946(03)00103-1*

128. Kumar, C.S., Ali, A., Manickavasagan, A., 2020. Health Benefits of Substituting Added Sugars with Fruits in Developing Value-Added Food Products: A Review. *International Journal of Nutrition, Pharmacology, Neurological Diseases* **10**(3), 75-90. *DOI:10.4103/ijnpnd.ijnpnd-34-20*

129. Kuras, M.J., Zielińska-Pisklak, M., Duszyńska, J., Jabłońska, J., 2020. Determination of the elemental composition and antioxidant properties of dates (*Phoenix dactyliferia*) originated from different regions. *Journal of food science and technology* **57**, 1-12. *https://doi.org/10.1007/s13197-020-04314-8*

130. Kwiatkowska, B., Bennett, J., Akunna, J., Walker, G.M., Bremner, D.H., 2011. Stimulation of bioprocesses by ultrasound. *Biotechnology advances* **29**(6), 768-780. *https://doi.org/10.1016/j.biotechadv.2011.06.005*

131. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *nature* **227**, 680-685.

132. Lemine, F.M.M., Samb, A., Zein el Abidine, O.B., Ahmed, M.V.O.M., Djeh, T.K.O., Boukhary, A.O.M.S.O., 2014. Assessment of physicochemical diversity in

fruit of Mauritanian date palm (*Phoenix dactylifera* L.) cultivars. *African Journal of Agricultural Research* **9**(28), 2167-2176. *DOI:10.5897/AJAR2013.8250*

133. Li, H., Deng, Z., Wu, T., Liu, R., Loewen, S., Tsao, R., 2012. Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. *Food chemistry* **130**(4), 928-936. *https://doi.org/10.1016/j.foodchem.2011.08.019*

134. Liu, R., Ye, Y., Qiang, L., Liao, X., Zhao, Y., 2008. The fragmentation pathway of the nucleosides under the electrospray ionization multi-stage mass spectrometry. *Life Sci. J* **5**(2), 37-40.

135. Ma, C., Yu, H., Gao, Y., Xu, W., Xu, T., Wang, L., Zhao, B., Zhang, Z., Xu, J., 2020. Operation parameters optimization of a hybrid dead-end/cross-flow forward osmosis system for microalgae dewatering by response surface methodology. *Process Safety and Environmental Protection* **143**, 14-24. *https://doi.org/10.1016/j.psep.2020.06.010*

136. Mac Graw H., 1797. Dairy substitutes, Encyclopedia of sci. and tech. 5th ed. New York, Mac Graw Hill publishers.

137. Mahmed, A.M., Hahidh, B., Aid, A., Yosef, H., Safaa, H., 2019. Sensory Evaluation of Jam Produced from Hearts of Palm Kestaweey Varieties of *Phoenix Dactylifera*, L. *Indian Journal of Public Health Research and Development* **10**(4), 1987-1990.

138. Mahmud, I., Mirghani, M.E., Al-Qubaisi, M., Yee, B., Ismail, N., Rasedee, A., Yusof, F., Shahabuddin, M., Kabbashi, N., Hashim, Y., 2019. Induction of apoptosis and cell cycle arrest in two human cancer A549 and HT29 cells lines by Barhi date palm kernels extracts and three isolated cytotoxic compounds. *Preprints. DOI:10.20944/preprints201906.0003.v1*

139. Maqsood, S., Adiamo, O., Ahmad, M., Mudgil, P., 2020. Bioactive compounds from date fruit and seed as potential nutraceutical and functional food ingredients. *Food chemistry* **308**, 125522. *https://doi.org/10.1016/j.foodchem.2019.125522*

140. Matini, S., Mortazavi, S.A., Sadeghian, A.R., Sharifi, A., 2020. Optimization of Ultrasound Assisted and Maceration Extraction of Bioactive Compounds of Sardasht Black Grape residue by using Response Surface Methodology. *Food Science and Technology* **17**, 147-158. DOI:10.29252/fsct.17.01.13

141. Medeiros, S.R.A., Oliveira, V.A.d., Oliveira, A.M.C.d., Araujo, M.L.H., Feitosa, J.P.d.A., Paula, R.C.M.d., Sousa, F.D.d., Moreira, A.C.d.O.M., Beserra, F.J.,

Moreira, R.d.A., 2020. Caesalpinia pulcherrima seed galactomannan on rheological properties of dairy desserts. *Ciência Rural* **50**(6). *https://doi.org/10.1590/0103-8478cr20190176*

142. Mena, P., Calani, L., Dall'Asta, C., Galaverna, G., García-Viguera, C., Bruni, R., Crozier, A., Del Rio, D., 2012. Rapid and comprehensive evaluation of (poly) phenolic compounds in pomegranate (*Punica granatum* L.) juice by UHPLC-MS^n. *Molecules* **17**, 14821-14840. *DOI:10.3390/molecules171214821*

143. Milardović, S., Iveković, D., Grabarić, B.S., 2006. A novel amperometric method for antioxidant activity determination using DPPH free radical. *Bioelectrochemistry* **68**(2), 175-180. *https://doi.org/10.1016/j.bioelechem.2005.06.005*

144. Mimouni, Y., 2015. Développement de produits diététiques hypoglycémiants à base de dattes molles variété «Ghars», la plus répandue dans la cuvette de Ouargla. Thèse doctorale, Université de Ouargla, Algérie. *https://bu.univouargla.dz/Theses%20DOCTORAT/Mimouni-Yamina-Doctorat.pdf*

145. Mirza, M.B., Elkady, A.I., Al-Attar, A.M., Syed, F.Q., Mohammed, F.A., Hakeem, K.R., 2018. Induction of apoptosis and cell cycle arrest by ethyl acetate fraction of *Phoenix dactylifera* L.(Ajwa dates) in prostate cancer cells. *Journal of ethnopharmacology* **218**, 35-44. *https://doi.org/10.1016/j.jep.2018.02.030*

146. Mishra, N., Gupta, E., Prasad, R., Singh, P., 2020. Formulation and optimization of malted barley based symbiotic beverage by response surface methodology. *Journal of Advances in Food Science and Technology* **7**(1), 12-20.

147. Mohd Basri, M.S., Mohd Jais, N., Sulaiman, A., Mohd Nor, M.Z., Abdul Karim Shah, N.N., Ariffin, S.H., 2020. Optimizing the Processing Factor and Formulation of Oat-Based Cookie Dough for Enhancement in Stickiness and Moisture Content Using Response Surface Methodology and Superimposition. *Processes* **8**, 797. *DOI:10.3390/pr8070797*

148. Moraes, M.N., Zabot, G.L., Prado, J.M., Meireles, M.A.A., 2013. Obtaining antioxidants from botanic matrices applying novel extraction techniques. *Food and Public Health* **3**(4), 195-214. *DOI:10.5923/j.fph.20130304.04*

149. Movahhedin, N., Zengin, G., Bahadori, M.B., Sarikurkcu, C., Bahadori, S., Dinparast, L., 2016. Ajuga chamaecistus subsp. scoparia (Boiss.) Rech. f.: A new source of phytochemicals for antidiabetic, skin-care, and neuroprotective uses. *Industrial Crops and Products* **94**, 89-96. *https://doi.org/10.1016/j.indcrop.2016.08.028*

150. Mushtaq, A., Roobab, U., Denoya, G.I., Inam-Ur-Raheem, M., Gullón, B., Lorenzo, J.M., Barba, F.J., Zeng, X.A., Wali, A., Aadil, R.M., 2020. Advances in green processing of seed oils using ultrasound-assisted extraction: A review. *Journal of Food Processing and Preservation*, e14740. *https://doi.org/10.1111/jfpp.14740*

151. Mrabet, A., Ferchichi, A., Chaira, N., Mohamed, B.S., 2008. Physico-chemical characteristics and total quality. *Pakistan Journal of Biological Sciences* **11**(7), 1003-1008.

152. Mrabet, A., Jiménez-Araujo, A., Fernández-Bolaños, J., Rubio-Senent, F., Lama-Muñoz, A., Sindic, M., Rodríguez-Gutiérrez, G., 2016. Antioxidant phenolic extracts obtained from secondary Tunisian date varieties (*Phoenix dactylifera* L.) by hydrothermal treatments. *Food chemistry* **196**, 917-924. *https://doi.org/10.1016/j.foodchem.2015.10.026*

153. Munier, P., 1961. Note sur le séchage et le conditionnement des dattes communes. *Fruits* **16**, 415-417.

154. Nasrolah, M., Honarvar, M., Bahrami, M.E., Zulfiqar, A., Bayat, M.H., Bahrami, F., Khanlarloo, H., 2019. The Occurrence of Acrylamide in Iranian Date Syrup. *Journal of Food Biosciences and Technology* **9**(1), 65-68.

155. Nieto-Mazzocco, E., Saldaña-Robles, A., Franco-Robles, E., Rangel-Contreras, A.K., Cerón-García, A., Ozuna, C., 2020. Optimization of sorghum, rice, and amaranth flour levels in the development of gluten-free bakery products using response surface methodology. *Journal of Food Processing and Preservation* **44**, e14302. *https://doi.org/10.1111/jfpp.14302*

156. Nitayavardhana, S., Rakshit, S.K., Grewell, D., Van Leeuwen, J., Khanal, S.K., 2008. Ultrasound pretreatment of cassava chip slurry to enhance sugar release for subsequent ethanol production. *Biotechnology and bioengineering* **101**(3), 487-496. *DOI:10.1002/bit.21922*

157. Nguyen Tram Anh, M., Van Hung, P., Thi Lan Phi, N., 2021. Optimized Conditions for Flavonoid Extraction from Pomelo Peel Byproducts under Enzyme-and Ultrasound-Assisted Extraction Using Response Surface Methodology. *Journal of Food Quality* **2021**. *https://doi.org/10.1155/2021/6666381*

158. Nongonierma, A.B., Cayot, P., Springett, M., Le Quéré, J.-L., Cachon, R., Voilley, A., 2007. Transfers of small analytes in a multiphasic stirred fruit yoghurt model. *Food Hydrocolloids* **21**(2), 287-296. *https://doi.org/10.1016/j.foodhyd.2006.04.007*

159. Norziah, M., Kong, S., Abd Karim, A., Seow, C., 2001. Pectin-sucrose-Ca^{2+} interactions: effects on rheological properties. *Food Hydrocolloids* **15**(4), 491-498. *https://doi.org/10.1016/S0268-005X(01)00089-3*

160. Obode, O.C., Adebayo, A.H., Li, C., 2020. Gas chromatography-mass spectrometry analysis and in vitro inhibitory effects of *Phoenix dactylifera* L. on key enzymes implicated in hypertension. *Journal of Pharmacy and Pharmacognosy Research* **8**(5), 475-490.

161. Olawoye, B., Gbadamosi, S.O., Otemuyiwa, I.O., Akanbi, C.T., 2020. Gluten-free cookies with low glycemic index and glycemic load: optimization of the process variables via response surface methodology and artificial neural network. *Heliyon* **6**(10), e05117. *https://doi.org/10.1016/j.heliyon.2020.e05117*

162. Oliveira, A., Alexandre, E.M., Coelho, M., Lopes, C., Almeida, D.P., Pintado, M., 2015. Incorporation of strawberries preparation in yoghurt: Impact on phytochemicals and milk proteins. *Food chemistry* **171**, 370-378. *https://doi.org/10.1016/j.foodchem.2014.08.107*

163. Oludemi, T., Barros, L., Prieto, M., Heleno, S.A., Barreiro, M.F., Ferreira, I.C., 2018. Extraction of triterpenoids and phenolic compounds from Ganoderma lucidum: optimization study using the response surface methodology. *Food and function* **9**, 209-226. *DOI:10.1039/c7fo01601hrsc.li/food-function*

164. Othmani, A., Jemni, M., Kadri, K., Amel, S., Artés, F., Al-Khayri, J.M., 2020. Preharvest fruit drop of date palm (*Phoenix dactylifera* L.) Cv. Deglet Nour at Kimri Stage: Development, physico-chemical characterization, and functional properties. *International Journal of Fruit Science* **20**(3), 414-432. *https://doi.org/10.1080/15538362.2019.1651241*

165. Orojloo, M., Orjloo, M., 2019. Evaluation of the Physical Characteristics and Nutritional Value of Five Varieties of Dates (*Phoenix dactylifera* L.) in Two Years of Storage. *ALKHAS; The Journal of Environment, Agriculture and Biological Sciences* **1**(1), 1-10.

166. Oyaizu, M., 1986. Studies on products of browning reaction. *The Japanese journal of nutrition and dietetics* **44**(6), 307-315.

167. Pandey, D.K., Kaur, P., 2018. Optimization of extraction parameters of pentacyclic triterpenoids from Swertia chirata stem using response surface methodology. *Biotechnology* **8**, 1-10. *https://doi.org/10.1007/s13205-018-1174-6*

168. Peerkhan, N., Nair, S., 2020. Optimization of wheat dextrin yogurt formulation using response surface methodology. *Journal of food science and technology*, 1-10. *https://doi.org/10.1007/s13197-020-04683-0*

169. Pinto, L.d.P.F., Silva, H.L., Kuriya, S.P., Maçaira, P.M., Cyrino Oliveira, F.L., Cruz, A.G., Esmerino, E.A., Freitas, M.Q., 2018. Understanding perceptions and beliefs about different types of fermented milks through the application of projective techniques: A case study using Haire's shopping list and free word association. *Journal of Sensory Studies* **33**, e12326. *DOI:10.1111/joss.12326*

170. Pongsumpun, P., Iwamoto, S., Siripatrawan, U., 2020. Response surface methodology for optimization of cinnamon essential oil nanoemulsion with improved stability and antifungal activity. *Ultrasonics Sonochemistry* **60**, 104604. *https://doi.org/10.1016/j.ultsonch.2019.05.021*

171. Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry* **269**, 337-341.

172. Prasad, W., Khamrui, K., Mandal, S., Badola, R., 2018. Effect of combination of essential oils on physicochemical and sensorial attributes of burfi in comparison with individual essential oil and BHA. *International journal of dairy technology* **71**(1), 810-819. *DOI:10.1111/1471-0307.12512*

173. Puri, R., Khamrui, K., Tomar, S., Lule, V., 2017. Effect of ingredients and processing parameters on the texture and microstructure of acid-heat coagulated dairy dessert. *Journal of Food Processing and Preservation* **41**, e12841. *DOI:10.1111/jfpp.12841*

174. Qadir, A., Shakeel, F., Ali, A., Faiyazuddin, M., 2020. Phytotherapeutic potential and pharmaceutical impact of *Phoenix dactylifera* (date palm): current research and future prospects. *Journal of food science and technology* **57**, 1191-1204. *https://doi.org/10.1007/s13197-019-04096-8*

175. Rahmani, A.H., Aly, S.M., Ali, H., Babiker, A.Y., Srikar, S., 2014. Therapeutic effects of date fruits (*Phoenix dactylifera*) in the prevention of diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour activity. *International journal of clinical and experimental medicine* **7** (3), 483-491.

176. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decol-

orization assay. *Free radical biology and medicine* **26**(9), 1231-1237. *https://doi.org/10.1016/S0891-5849(98)00315-3*

177. Rohilla, S., Mahanta, C.L., 2021. Optimization of extraction conditions for ultrasound-assisted extraction of phenolic compounds from tamarillo fruit (*Solanum betaceum*) using response surface methodology. *Journal of Food Measurement and Characterization*, 1-11. *https://doi.org/10.1007/s11694-020-00751-3*

178. Saddi, A.A., Mohamed, A.M., Shaikh, A.M., 2018. Prophylactic mechanisms of Cucumis melo var. flexuosus and *Phoenix dactylifera* fruit extracts against diabetic cardiomyopathy in streptozotocin induced diabetic rats. *Pakistan journal of pharmaceutical sciences* **31**, 699-707.

179. Sah, B.N.P., Vasiljevic, T., McKechnie, S., Donkor, O., 2015. Effect of refrigerated storage on probiotic viability and the production and stability of antimutagenic and antioxidant peptides in yogurt supplemented with pineapple peel. *Journal of dairy science* **98** (9), 5905-5916. *https://doi.org/10.3168/jds.20159450*

180. Şahin, S., Şamlı, R., 2013. Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. *Ultrasonics Sonochemistry* **20**(1), 595-602. *https://doi.org/10.1016/j.ultsonch.2012.07.029*

181. Sahu, C., Patel, S., 2020. Optimization of maize–millet based soy fortified composite flour for preparation of RTE extruded products using D-optimal mixture design. *Journal of food science and technology*, 1-10. *https://doi.org/10.1007/s13197-020-04771-1*

182. Saleh, E.A., Tawfik, M.S., Abu-Tarboush, H.M., 2011. Phenolic contents and antioxidant activity of various date palm (*Phoenix dactylifera* L.) fruits from Saudi Arabia. *Food and Nutrition Sciences* **2**(10), 1134-1141. *DOI:10.4236/fns.2011.210152*

183. Salem, G.A., Shaban, A., Diab, H.A., Elsaghayer, W.A., Mjedib, M.D., Hnesh, A.M., Sahu, R.P., 2018. *Phoenix dactylifera* protects against oxidative stress and hepatic injury induced by paracetamol intoxication in rats. *Biomedicine and Pharmacotherapy* **104**, 366-374. *https://doi.org/10.1016/j.biopha.2018.05.049*

184. Sallon, S., Cherif, E., Chabrillange, N., Solowey, E., Gros-Balthazard, M., Ivorra, S., Terral, J.F., Egli, M., Aberlenc, F., 2020. Origins and insights into the historic Judean date palm based on genetic analysis of germinated ancient seeds and morphometric studies. *Science advances* **6**(6), eaax0384. *DOI:10.1126/sciadv.aax0384*

185. Sangamithra, A., Sivakumar, V., Kannan, K., John, S.G., 2015. Foam-mat drying of muskmelon. *International Journal of Food Engineering* **11**(1), 127-137. *DOI:10.1515/ijfe-2014-0139*

186. Sarfarazi, M., Jafari, S.M., Rajabzadeh, G., Galanakis, C.M., 2020. Evaluation of microwave-assisted extraction technology for separation of bioactive components of saffron (*Crocus sativus* L.). *Industrial Crops and Products* **145**, 111978. *https://doi.org/10.1016/j.indcrop.2019.111978*

187. Saunders, A., 2011. Ice Cream and Desserts-Dairy Desserts. *Encyclopedia of Dairy Sciences (2nd), Academic Press, San Diego, CA* 905-912. *https://doi.org/10.1016/B978-0-12-374407-4.00246-6*

188. Sayah, Z., 2010. Etude comparative des caractéristiques physico-chimiques et biochimiques des dattes de la cuvette de Ouargla. *Annales des Sciences et Technologie*, **2**, 87-92.

189. Sayas-Barberá, E., Martín-Sánchez, A.M., Cherif, S., Ben-Abda, J., Pérez-Álvarez, J.Á., 2020. Effect of date (*Phoenix dactylifera* L.) pits on the shelf life of beef burgers. *Foods* **9**(1), 102. *DOI:10.3390/foods9010102*

190. Schieber, A., Keller, P., Streker, P., Klaiber, I., Carle, R., 2002. Detection of isorhamnetin glycosides in extracts of apples (Malus domestica cv."Brettacher") by HPLC/PDA and HPLC/APCI/MS/MS. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques* **13**, 87-94. *DOI:10.1002/pca.630*

191. Seerangurayar, T., Manickavasagan, A., Al-Ismaili, A.M., Al-Mulla, Y.A., 2018. Effect of carrier agents on physicochemical properties of foam-mat freeze-dried date powder. *Drying Technology* **36**(11), 1292-1303. https://doi.org/10.1080/07373937.2017.1400557

192. Setyaningsih, W., Saputro, I., Palma, M., Barroso, C., 2015. Optimisation and validation of the microwave-assisted extraction of phenolic compounds from rice grains. *Food chemistry* **169**, 141-149. *https://doi.org/10.1016/j.foodchem.2014.07.128*

193. Sevda, S., Singh, A., 2020. Mathematical and Statistical Applications in Food Engineering. *CRC Press.*

194. Shahdadi, F., Mirzaei, H., Garmakhany, A.D., 2015. Study of phenolic compound and antioxidant activity of date fruit as a function of ripening stages and drying process. *Journal of food science and technology* **52**, 1814-1819. *https://doi.org/10.1007/s13197-013-1177-6*

195. Siddeeg, A., Zeng, X.A., Ammar, A.F., Han, Z., 2019. Sugar profile, volatile compounds, composition and antioxidant activity of Sukkari date palm fruit. *Journal*

of food science and technology **56**, 754-762. *https://doi.org/10.1007/s13197-018-3534-y*

196. Sifaoui, I., Mecha, E., Silva, A., Chammem, N., Mejri, M., Abderabba, M., Bronze, M.R., 2016. Optimized extraction of antioxidants from olive leaves using augmented simplex centroid design. *Analytical Letters* **49**(9), 1323-1333. *https://doi.org/10.1080/00032719.2015.1104320*

197. Singh, R., Bhunia, P., Dash, R.R., 2019a. Optimization of bioclogging in vermifilters: a statistical approach. *Journal of Environmental Management* **233**, 576-585. *https://doi.org/10.1016/ j.jenvman.2018.12.065*

198. Singh, R., Bhunia, P., Dash, R.R., 2019b. Optimization of organics removal and understanding the impact of HRT on vermifiltration of brewery wastewater. *Science of The Total Environment* **651**(1), 1283-1293. *https://doi.org/10.1016/ j.scitotenv.2018.09.307*

199. Sobeh, M., ElHawary, E., Peixoto, H., Labib, R.M., Handoussa, H., Swilam, N., El-Khatib, A.H., Sharapov, F., Mohamed, T., Krstin, S., 2016. Identification of phenolic secondary metabolites from Schotia brachypetala Sond.(Fabaceae) and demonstration of their antioxidant activities in Caenorhabditis elegans. *Peer Journal* **4**, e2404. *https://doi.org/10.7717/peerj.2404*

200. Sokeng, A.J.T., Sobolev, A.P., Di Lorenzo, A., Xiao, J., Mannina, L., Capitani, D., Daglia, M., 2019. Metabolite characterization of powdered fruits and leaves from Adansonia digitata L.(Baobab): A multi-methodological approach. *Food chemistry* **272**, 93-108. *https://doi.org/10.1016/j.foodchem.2018.08.030*

201. Soliman, M.S., Abd-Allah, F.I., Hussain, T., Saeed, N.M., El-Sawy, H.S., 2018. Date seed oil loaded niosomes: development, optimization and anti-inflammatory effect evaluation on rats. *Drug development and industrial pharmacy* **44**, 1185-1197. *https://doi.org/10.1080/03639045.2018.1438465*

202. Sudha, T., Divya, G., Sujaritha, J., Duraimurugan, P., 2017. Review of experimental design in analytical chemistry. *Journal of Pharmaceutical Research* **7**(8), 550-565.

203. Sumampouw, G.A., Jacobsen, C., Getachew, A.T., 2021. Optimization of phenolic antioxidants extraction from Fucus vesiculosus by pressurized liquid extraction. *Journal of Applied Phycology*, 1-13. *https://doi.org/10.1007/s10811-020-02362-3*

204. Sun, Y., Wang, W., 2008. Ultrasonic extraction of ferulic acid from Ligusticum chuanxiong. *Journal of the Chinese Institute of Chemical Engineers* **39**(6), 653-656. *https://doi.org/10.1016/j.jcice.2008.05.012*

205. Swamy, G.J., Sangamithra, A., Chandrasekar, V., 2014. Response surface modeling and process optimization of aqueous extraction of natural pigments from Beta vulgaris using Box–Behnken design of experiments. *Dyes and Pigments* **111**, 64-74. *https://doi.org/10.1016/j.dyepig.2014.05.028*

206. Szwajgier, D., Gustaw, W., 2015. The addition of malt to milk-based desserts: Influence on rheological properties and phenolic acid content. *LWT-Food Science and Technology* **62** (1), 400-407. *https://doi.org/10.1016/j.lwt.2015.01.028*

207. Taleb, H., Maddocks, S.E., Morris, R.K., Kanekanian, A.D., 2016. The antibacterial activity of date syrup polyphenols against S. aureus and E. coli. *Frontiers in microbiology* **7**, 198. *https://doi.org/10.3389/fmicb.2016.00198*

208. Tanase, C., Cosarca, S., Muntean, D.L., 2019. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. *Molecules* **24**, 1182. *DOI:10.3390/molecules24061182*

209. Tappy, L., Rosset, R., 2017. Fructose metabolism from a functional perspective: implications for athletes. *Sports medicine* **47**(1), 23-32. *DOI:10.1007/s40279-017-0692-4*

210. Thangaraj, P., 2018. Medicinal Plants: Promising Future for Health and New Drugs. *CRC Press: Boca Raton*, *1st ed*, *USA*, 382.

211. Torres, F., Esmerino, E., Carr, B.T., Ferrão, L., Granato, D., Pimentel, T., Bolini, H., Freitas, M., Cruz, A., 2017. Rapid consumer-based sensory characterization of requeijão cremoso, a spreadable processed cheese: Performance of new statistical approaches to evaluate check-all-that-apply data. *Journal of dairy science* **100**, 6100-6110.

212. Tran, Q.T., Vu Thi, T.L., Do, T.L., Pham Thi, H.M., Hoang Thi, B., Chu, Q.T., Lai Phuong, P.T., Do, H.N., Hoang Than, H.T., Ta Thi, T.T., 2020. Optimization of Microwave-Assisted Extraction Process of Callicarpa candicans (Burm. f.) Hochr Essential Oil and Its Inhibitory Properties against Some Bacteria and Cancer Cell Lines. *Processes* **8**, 173. *DOI:10.3390/pr8020173*

213. Trigueros, L., Sayas-Barberá, E., Pérez-Álvarez, J., Sendra, E., 2012. Use of date (Phoenix dactylifera L.) blanching water for reconstituting milk powder: Yogurt

manufacture. *Food and Bioproducts Processing* **90** (2), 506-514. *https://doi.org/10.1016/j.fbp.2011.10.001*

214. Türker, D.A., Saraç, M.G., Doğan, M., 2020. Influence of sucrose reduction and starch type on bulk and powder properties of ready-to-use powdered dessert. *European Food Research and Technology*, 1-12. *https://doi.org/10.1007/s00217-020-03638-1*

215. Turki, M., Barbosa-Pereira, L., Bertolino, M., Essaidi, I., Ghirardello, D., Torri, L., Bouzouita, N., Zeppa, G., 2020. Physico-chemical characterization of Tunisian canary calm (Phoenix canariensis Hort. Ex Chabaud) dates and evaluation of their addition in biscuits. *Foods* **9**, 353-386. *DOI:10.1007/978-94-017-9707-8-15*

216. Vilkhu, K., Mawson, R., Simons, L., Bates, D., 2008. Applications and opportunities for ultrasound assisted extraction in the food industry—A review. *Innovative Food Science and Emerging Technologies* **9**(2), 161-169. *https://doi.org/10.1016/j.ifset.2007.04.014*

217. Wang, T., Guo, N., Wang, S.X., Kou, P., Zhao, C.J., Fu, Y.J., 2018. Ultrasound-negative pressure cavitation extraction of phenolic compounds from blueberry leaves and evaluation of its DPPH radical scavenging activity. *Food and Bioproducts Processing* **108**, 69-80. *https://doi.org/10.1016/j.fbp.2018.01.003*

218. Weremfo, A., Adulley, F., Adarkwah-Yiadom, M., 2020. Simultaneous Optimization of Microwave-Assisted Extraction of Phenolic Compounds and Antioxidant Activity of Avocado (*Persea americana* Mill.) Seeds Using Response Surface Methodology. *Journal of Analytical Methods in Chemistry* **2020**, 1-11. *https://doi.org/10.1155/2020/7541927*

219. Xiao, Y., Liu, L., Bian, J., Yan, C., Ye, L., Zhao, M., Huang, Q., Wang, W., Liang, K., Shi, Z., 2018. Identification of multiple constituents in shuganjieyu capsule and rat plasma after oral administration by ultra-performance liquid chromatography coupled with electrospray ionization and ion trap mass spectrometry. *Acta Chromatographica* **30**, 95-102.

220. Xiaokang, W., Lyng, J.G., Brunton, N.P., Cody, L., Jacquier, J.C., Harrison, S.M., Papoutsis, K., 2020. Monitoring the effect of different microwave extraction parameters on the recovery of polyphenols from shiitake mushrooms: Comparison with hot-water and organic-solvent extractions. *Biotechnology Reports* **27**, e00504. *https://doi.org/10.1016/j.btre.2020.e00504*

221. Yahmed, N.B., Dauptain, K., Lajnef, I., Carrere, H., Trably, E., Smaali, I., 2021. New sustainable bioconversion concept of date by-products (*Phoenix dactylifera*

L.) to biohydrogen, biogas and date-syrup. *International journal of hydrogen energy* **46**(1), 297-305. *https://doi.org/10.1016/j.ijhydene.2020.09.203*

222. Yeddes, W., Djebali, K., Wannes, W.A., Horchani-Naifer, K., Hammami, M., Younes, I., Tounsi, M.S., 2020. Gelatin-chitosan-pectin films incorporated with rosemary essential oil: Optimized formulation using mixture design and response surface methodology. *International Journal of Biological Macromolecules* **154**, 92-103. *https://doi.org/10.1016/j.ijbiomac.2020.03.092*

223. Yolmeh, M., Jafari, S.M., 2017. Applications of response surface methodology in the food industry processes. *Food and Bioprocess Technology* **10**, 413-433. *DOI:10.1007/s11947-016-1855-2*

224. Younas, A., Naqvi, S.A., Khan, M.R., Shabbir, M.A., Jatoi, M.A., Anwar, F., Inam-Ur-Raheem, M., Saari, N., Aadil, R.M., 2020. Functional food and nutra-pharmaceutical perspectives of date (*Phoenix dactylifera* L.) fruit. *Journal of Food Biochemistry* **44**, e13332. *https://doi.org/10.1111/jfbc.13332*

225. Zarbakhsh, S., Rastegar, S., 2019. Influence of postharvest gamma irradiation on the antioxidant system, microbial and shelf life quality of three cultivars of date fruits (*Phoenix dactylifera* L.). *Scientia Horticulturae* **247**, 275-286. *https://doi.org/10.1016/j.scienta.2018.12.035*

226. Zihad, S.N.K., Uddin, S.J., Sifat, N., Lovely, F., Rouf, R., Shilpi, J.A., Sheikh, B.Y., Göransson, U., 2021. Antioxidant properties and phenolic profiling by UPLC-QTOF-MS of Ajwah, Safawy and Sukkari cultivars of date palm. *Biochemistry and Biophysics Reports* **25**, 100909. *https://doi.org/10.1016/j.bbrep.2021.100909*

227. Zineb, G., Boukouada, M., Djeridane, A., Saidi, M., Yousfi, M., 2012. Screening of antioxidant activity and phenolic compounds of various date palm (*Phoenix dactylifera*) fruits from Algeria. *Mediterranean journal of nutrition and metabolism* **5**, 119-126. *DOI:10.1007/s12349-011-0082-7*

228. Zou, Y., Chen, X., Yang, W., Liu, S., 2011. Response surface methodology for optimization of the ultrasonic extraction of polysaccharides from Codonopsis pilosula Nannf. var. modesta LT Shen. *Carbohydrate Polymers* **84**(1), 503-508. *https://doi.org/10.1016/j.carbpol.2010.12.013*

Annexes

Annex 1. Calibration curves

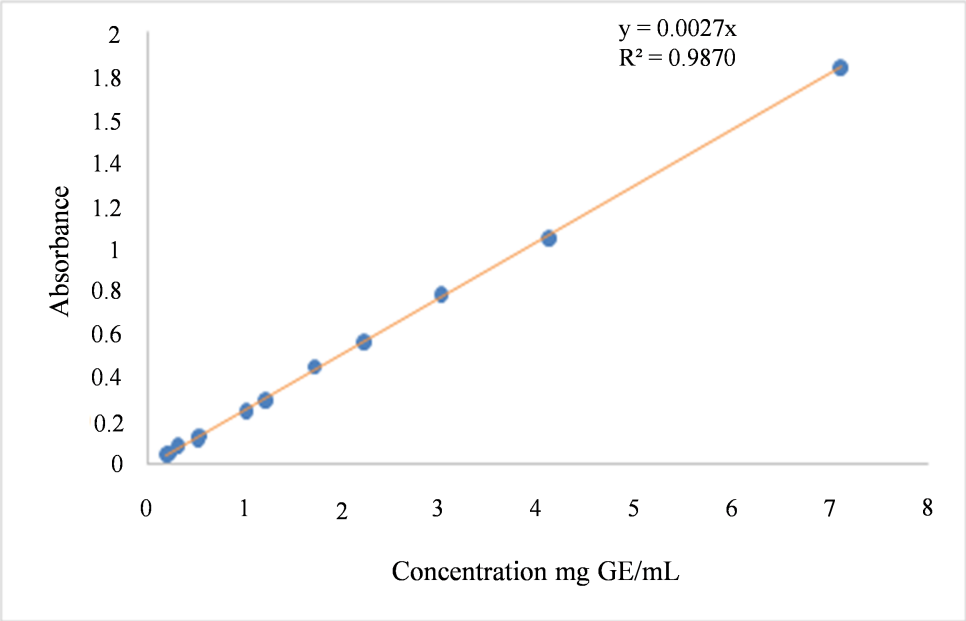


Figure 1. Glucose calibration curve.

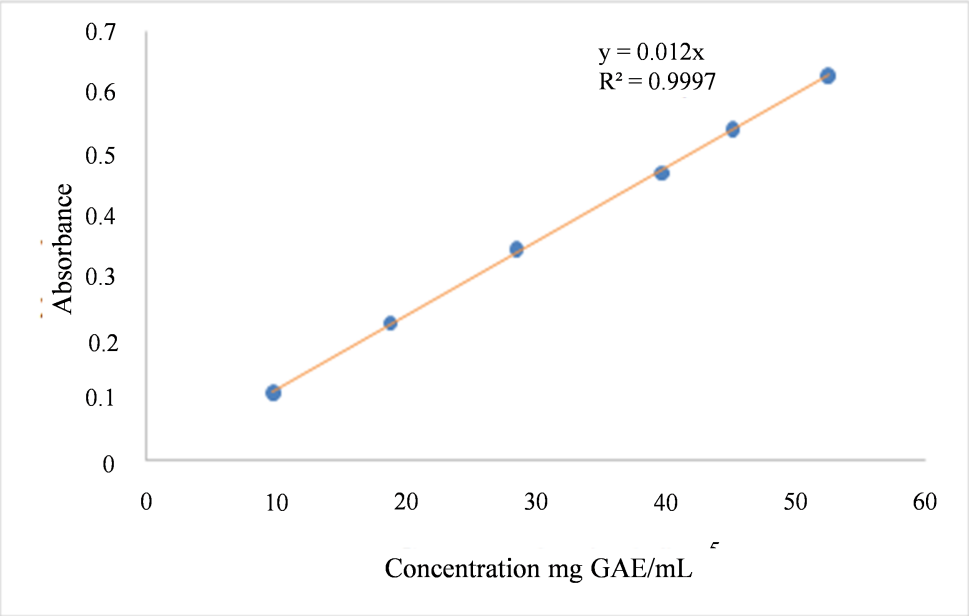


Figure 2. Gallic acid calibration curve.

Annex 2. Sensory evaluation questionnaire

UNIVERSITE ABDERRAHMANE MIRA DE BEJAIA
Laboratoire de Biochimie, Biophysique, Biomathématiques et Scientométrie

Evaluation sensorielle des crèmes desserts au sirop et au poudre de dattes

Age : ..... Sexe : F ou H Profession : .....

Cinq échantillons de crèmes desserts préparés à base de sirop et de poudre de datte à différentes concentrations vous sont présentés (sans aucun produit chimique). Veuillez évaluer les produits dans l'ordre de présentation et répondre pour tous les critères (texture, couleur, saveur ...) en attribuant une note de 1 a 5 selon votre préférence pour chaque échantillon.
N.B: Rincer la bouche avec de l'eau avant de passer d'un échantillon à un autre.

I) La couleur vous l'appréciez :
5 : très bien
4 : bien
3 : moyen
2 : peu
1 : pas du tout

A	B	C	D	E	F

II) L'odeur vous l'appréciez :
5 : très bien
4 : bien
3 : moyen
2 : peu
1 : pas du tout

A	B	C	D	E	F

III) La texture vous l'appréciez :
5 : très bien
4 : bien
3 : moyen
2 : peu
1 : pas du tout

A	B	C	D	E	F

IV) La granulosité vous l'appréciez :

5 : très bien
4 : bien
3 : moyen
2 : peu
1 : pas du tout

A	B	C	D	E	F

V) La sucrosité vous l'appréciez :

5 : très bien
4 : bien
3 : moyen
2 : peu
1 : pas du tout

A	B	C	D	E	F

VII) La saveur vous l'appréciez :

5 : très bien
4 : bien
3 : moyen
2 : peu
1 : pas du tout

A	B	C	D	E	F

VIII) Attribuez pour chaque échantillon de crèmes desserts une note de préférence entre 1 et 9, sachant que 1 correspond à l'échantillon le moins préféré et 9 au plus préféré :

A	B	C	D	E	F

Merci de participer à cette évaluation

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RESEARCH ARTICLE

Syrup from Common Date Variety (*Phoenix dactylifera* L.): Optimization of Sugars Extraction and their Quantification by High Performance Liquid Chromatography

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Abstract: ***Background*:** In Algeria, important quantities of secondary date variety (*Phoenix dactylifera* L.) are generated in each campaign; their chemical composition is similar to that of commercial dates. The present work aims to valorize this common date variety (Degla-Beida) which is often poorly exploited.

***Methods*:** In this context, we tried to prepare syrup from the secondary date variety and evaluate the effect of conventional extraction (CE) or water bath extraction (WBE) and alternative extraction (microwaves assisted extraction (MAE) and ultrasound-assisted extraction (UAE)) on its total sugar content (TSC), using response surface methodology (RSM). Then, the analysis of individual sugars was performed by high performance liquid chromatography (HPLC).

***Results*:** Maximum predicted TSC recoveries under the optimized conditions for MAE, UAE and CE were 233.248 ± 3.594 g/l, 202.889 ± 5.797g/land 233.535 ± 5.412g/l, respectively, which were close to the experimental values: 233.796 ± 1.898 g/l; 202.037 ± 3.401 g/land 234.380 ± 2.425 g/l. HPLC analysis revealed high similarity in the sugar composition of date juices obtained by MAE (60.11% sucrose, 16.64% glucose and 23.25% fructose) and CE (50.78% sucrose, 20.67% glucose and 28.55% fructose), although a large difference was detected for that obtained by UAE (0.00% sucrose, 46.94% glucose and 53.06% fructose).

***Conclusion*:** Microwave-assisted extraction was the best method for the preparation of date syrup with an optimal recovery of total sugar content. However, ultrasound-assisted extraction was the best one for the preparation of date syrup with high content of reducing sugars.

Keywords: Dates, extraction methods, HPLC analysis, RSM, sugars, syrup.

1. INTRODUCTION

The analysis and exploitation of bioactive substances involve their extraction from the cell matrix. Indeed, the latter are released from the vacuolar structures in which they are located, either by rupture of the plant tissue or by simple diffusion [1]. The best extraction technique is that which extracts more substances, while preserving their structure and taking a short time. The conventional extraction process is commonly used [2], although recently many non-conventional methods, which are rapid and automated, have been used, *e.g.* supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) [3]. Many reports have confirmed that ultrasound and microwave extractions are the fastest methods for extracting bioactive components from vegetable matrices [2, 4].

Since 7000 years, dates have played an important role in human life [5]. They are the fundamental fruit of oasis ecosystems, and have been an important crop in the Sahara regions [5-7]. The industrialization and transformation of dates are gradually increasing due to their richness in essential nutrients [5]. The date and its by-products play an important role in the economy of producing countries [6, 8]. During the Ramadan period, dates are the main fruits for the Algerians and especially for the inhabitants of the Sahara [9]. They are used with fermented milk to break the fast because they are considered an important source of nutrients and energy.

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Palm date fruits and syrups are used to treat liver diseases in traditional medicine [10]. Because of their richness in potassium and its low sodium content, they are considered as a desirable food for hypertensive patients who are advised to consume low sodium diets [11]. The regular consumption of dates is beneficial for the treatment of cough, rheumatism, burning sensation, nephropathy, gastropathy, and sexual debility. Therefore, they are prescribed for gastroenteritis, respiratory diseases, asthma, chest complaints, fevers and high blood pressure [10]. Epidemiological studies have also reported their effect on reducing the risk of several neurodegenerative diseases, such as tumors and mutagens. They are also used to stimulate the immune system of women after giving birth [12].

The global production of date fruits exceeds 7 million metric tons annually in the world. Algeria is the second producer in the world with an annual production of 720 000 tons [13], and the first producer in the Arab Maghreb union [14]. Cultivars with a low commercial quality, known as common cultivars, are discarded and account for approximately 30% of the Algerian production [15], although second-grade dates (with a hard texture) contain the same levels of sugar, fiber and total phenols [7].

Date syrup (date honey), is a dark sweet syrup obtained from the date extract [6] and it is made with different varieties of dates, preferably those of secondary quality [16, 17]. It is a highly concentrated liquid [18], which is a naturally inverted sugar, with equal proportions of glucose and fructose, and a small amount of sucrose, which under the effect of temperature and pH conditions (acid medium) can be inverted to a simple sugar [16]. It is consumed alone as honey, or used as an ingredient in food recipes such as: ice cream, beverages, confectionery, pancakes and waffles.

The chemical composition and nutritional value of date syrup have been well studied [6, 8, 19]. Date syrup is rich in carbohydrates and minerals, saccharides, amino and organic acids, polyphenols and carotenoids [6, 9]. It can replace synthetic sweeteners [17], which during the manufacturing processes can lead to the formation of toxic products [20]. As it is a natural invert sugar, toxic chemicals are not produced after its manufacturing processes (pressing, extraction at low and high temperature) [16].

The main objective of this work is to optimize the method of preparation of syrup with high total sugar content (TSC), from low quality and hard texture (Degla-Beida) dates. To this end, three methods were proposed: microwave method (MM), ultrasound method (UM) and conventional or water bath method (CM or WBM). Response surface methodology (RSM) was used to evaluate the factors affecting the sugar yield in both methods. The optimized extracts were then analyzed for their individual sugar composition using HPLC.

2. MATERIAL AND METHODS

2.1. Chemicals and Sample Treatment

All the chemicals used in this study were purchased from Sigma-Aldrich (represented by Algerian Chemical Society, Setif, Algeria). The date used was a common variety called Degla-Beida, of dry nature and hard texture, from Biskra (Algeria) at the final stage of fruit ripeness. The samples were washed with tap water followed by distilled water, pitted and deposited in a drying oven at 40°C until an unvarying weight and then grounded into a fine powder; this powder was passed through a standard 125 µm sieve and only the fraction with particle size <125 µm was used. The powder was stored in the dark until use. The choice of this variety is justified by its relative abundance in Algeria, low-market value, long-term conservation for its dry nature and by its taste and nutritional quality (source of energy), especially represented by its sugar content.

2.2. Preparation of Date Juices

Three extraction methods were used for the preparation of date juices: microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and conventional extraction (CE).

To prepare date syrups, the obtained juices were concentrated at 70°C using an oven until having syrups at 80°Brix.

2.2.1. Microwave Assisted Extraction (MAE)

A microwave oven (MAXMOS23S, Maxipower, China) working at 2450 MHz frequency with cavity dimensions of 28.1cm (H) × 48.3 cm (W) × 38.7 cm (D) was used. Ten grams (10 g) of date powder was mixed with 60, 70, 80, 90, 100, 120 and 140 ml of distillated water. The samples were treated under microwave irradiations in an intermittent way, *i.e*. irradiation-cooling-irradiation at power input of 100, 300, 500, 700 and 900 W with fixed irradiation and cooling time of 0.30, 0.45, 1, 2, 3, 4 and 5 min. After irradiation, the extract was filtered through a Whatman filter paper (n° 4), and centrifuged (NF200, NÜVE, Turkey) at 4000 rpm for 15min, and then adjusted to 100 ml with distilled water. Finally, the extract was stored at 4°C until use.

2.2.2. Ultrasound Assisted Extraction (UAE)

Date powder (10g) was immersed into 60, 70, 80, 90, 100, 120 and 140 ml of distilled water. The extraction process was performed using an ultrasonic cleaner (2510E-DTH, Mexico, USA), with working frequency fixed at 42 kHz and different ultrasonic temperatures: 30, 40, 50, 60 and 69°C. After 15, 30, 45, 60, 90 min, the extract was filtered through Whatman filter paper (n° 4), centrifuged (NF200, NÜVE, Turkey) at 4000 rpm for 15 min, and then adjusted to 100 ml with distilled water. At the end, the extract was stored at 4°C, until use.

2.2.3. Conventional Extraction (CE) or Water Bath Extraction (WBE)

Conventional extraction was carried out as follows: 10g of date powder was extracted for 1, 30, 60, 90, 120 and 180 min using a thermostatic water bath shaker (WNB22, Memmert, Germany) with 60, 70, 80, 90, 100, 120 and 140 ml of distilled water at different shaking speed (30, 50, 70, 90 and 110rpm, respectively) and different temperatures (40, 50, 60, 70, 80, 90 and 95°C, respectively). The extract was filtered

through Whatman filter paper (n° 4), centrifuged (NF200, NÜVE, Turkey) at 4000 rpm for 15 min, and then adjusted to 100 ml with distilled water. The obtained extract was stored (4°C) until use.

2.3. Determination of Total Sugar Content (TSC)

The most commonly used method to measure carbohydrate concentrations is the anthrone method [21]. Following the method described by Hanson and Phillips, (1981) [22], briefly, 400 µl of the extract (diluted in distilled water) was placed in the test tube, 2 ml of anthrone reagent was added and the solution was mixed. After incubation at 100°C for 10 min, the absorbance of the sample was measured at 625 nm against a blank (made as reported for the sample but with 400 µl of distilled water), by using a UV-vis Spectrophotometer (SpectroScan 50, UK). Glucose was used as standard for the calibration curve to express the TSC concentration of the sample as mg Equi. Glu/ml.

2.4. Identification of Sugars From Date Syrups by HPLC

High performance liquid chromatography was used for the analysis of sugars (fructose, glucose and sucrose) from the optimized juices. The separation was carried out on a cationic (Ca^{++}) or cation exchange column, the mobile phase (the eluent) was the distilled water and the detector was a Refractive Index Detector (RID). Sugars were extracted from different samples (0.1g) by stirring for 10 min in a 100 ml flask with 20 ml of distilled water, and then adjusted to 1.5ml. The samples were filtered through membrane filters (0.45 µm) and analyzed by liquid chromatography (LC). The results are expressed in percentages (%) by the software calculation of the area of each peak. The molecules of glucose, fructose and sucrose were characterized by their retention time (by comparison with their respective standards).

2.5. Experimental Design and Statistical Analysis

Response surface methodology (RSM) was applied to determine the optimal processing conditions generated by the statistical software package JMP (10.0.0 version, SAS Institute, USA). Three extraction methods with different processing variables including: X_1-Microwave power, X_2-Irradiation time, and X_3-Liquid/solid ratio for MAE; X_1-Ultrasonic temperature, X_2-Ultrasonic treatment time and X_3-Liquid/solid ratio for UAE and X_1-Temperature, X_2-Treatment time, X_3-Liquid/solid ratio and X_4-Shaking speed for CE, were chosen based on the results of the single factor experiments (Table **1**). Then, the effects of these key variables were investigated using Box-Behnken experimental design (BBD) in RSM to develop a response surface quadratic model for describing the extraction process. Then the yield of TSC (Y) was taken as the response of the design experiments. According to this design, a total of 15 experiments were performed for MAE and UAE; however, 27 experiments were performed for WBE (Table **2**) containing three replicates at the center point. The selected variables were coded according to the following equation:

$$x_i = X_i - X_{0i} / \Delta X \quad (1)$$

where x_i and Xi are the least coded dimension and the actual values for its independent variables, respectively, X_0 is the actual value for the independent variable at the center point, and ΔX is the step change value.

The regression analysis of the experimental data was performed to establish the empirical second order polynomial models, shown in Eq 2:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X^2 + \sum_{ij}^{k} \beta_{ij} X_i X_j + E \quad (2)$$

where Y is the measured response variable, which is in our case the total sugar content (TSC), $ß_0$ is a constant, $ß_i$ is the linear coefficient (main effect), $ß_{ii}$ is the quadratic coefficient, $ß_{ij}$ is the two factor interaction coefficients, and X_i, X_j are the independent variables.

Each extraction trial and all the analyses were carried out in triplicate and all the data in this paper were reported as means ± standard deviation (SD). The influence of each factor on the TSC yield in the single factor experiment for the MAE, UAE and CE was statistically assessed by ANOVA analysis of variance and Tukey's post hoc test with 95% confidence level using XLSTAT Release 10 (Addinsoft, Paris, France).

3. RESULTS AND DISCUSSION

Before carrying out the BBD approach, preliminary experiments were undertaken to select the lower, middle and upper level (+1, 0, -1) for each factor. The results of the preliminary experiments for the BBD are shown in Table **1**. Distilled water was then selected as the solvent for the RSM trials and it was used for all the extraction methods.

3.1. Microwave Assisted Extraction (MAE)

3.1.1. Effect of Independent Variables on TSC Yield

Low microwave power reduces extraction efficiency. However, too much power results in wasted energy; therefore, the optimal microwave power should be determined. TSC significantly increased when the microwave power increased from 100 W to 500 W, whereas the other extraction parameters were constant (1 min irradiation time and 10:1 ml/g liquid/solid ratio). TSC recovery was parabolic with a maximum value at 500 W. However, as the microwave power continued to increase up to 900 W, TSC decreased. Based on these results, the microwave power range 300-700 W was selected for the RSM trials and 500 W was fixed for the next single factor experiments on the influence of microwave irradiation time.

Extraction time is an important parameter in the extraction of phytochemicals. It has two phases: 1) the dissolution of soluble components on the surface of the sample particles, and 2) the mass transfer of the solute from the plant matrix into the solvent by diffusion and osmotic processes [23]. When the contact time between the solvent and the sample is longer, its absorption is improved, and the permeability of the cell wall is increased, which allows the solvent to penetrate the plant material in order to accelerate the release of

Table 1. Results of single-factor experiments of MA, UA and WB extractions.

MAE						UAE						WBE							
Power		Time		Ratio		T°		Time		Ratio		T°		Time		Ratio		Shaking Speed	
W	TSC (g/l)	s	TSC (g/l)	ml/g	TSC (g/l)	°C	TSC (g/l)	min	TSC (g/l)	ml/g	TSC (g/l)	°C	TSC (g/l)	min	TSC (g/l)	ml/g	TSC (g/l)	rpm	TSC (g/l)
100	104 ±5^{c}	30	110 ±3^{d}	6	73, ±9^{d}	30	33 ±3^{c}	15	103 ±4^{c}	6	81 ±4^{c}	40	144 ±5^{b}	1	151 ±3^{d}	6	152 ±4^{c}	30	156 ±10^{b}
300	190 ±3^{b}	45	155 ±8^{b}	7	111 ±7^{c}	40	92 ±7 ^{b}	30	131 ±2^{ab}	7	94 ±4^{bc}	50	168 ±8^{b}	30	217 ±8^{ab}	7	175 ±7^{bc}	50	162 ±10^{b}
500	220 ±1^{a}	60	220 ±1^{a}	8	117 ±5^{c}	50	108 ±10^{b}	45	153 ±6^{bc}	8	120 ±5^{abc}	60	180 ±8^{b}	60	229 ±3^{b}	8	182 ±5^{ab}	70	177 ±7^{ab}
700	175 ±4^{b}	120	230 ±1^{a}	9	160 ±6^{b}	60	131 ±10^{a}	60	196 ±6^{a}	9	154 ±6^{ab}	70	189 ±4^{ab}	90	174 ±6^{bc}	9	205 ±3^{ab}	90	195 ±6^{ab}
900	118 ±4^{c}	180	205 ±5^{a}	10	182 ±8^{a}	69	131 ±10^{a}	75	165 ±5^{a}	10	190 ±9^{a}	80	202 ±5^{ab}	120	160 ±4^{cd}	10	216 ±4^{a}	110	229 ±3^{a}
		240	156 ±7^{c}	12	184 ±6^{a}			90	119 ±7^{bc}	12	191 ±7^{a}	90	217 ±8^{a}	180	155 ±6^{cd}	12	215 ±6^{a}		
		300	132 ±4^{c}	14	185 ±7^{a}					14	190 ±5^{a}								

Values are mean ± 95 % confidence interval.
Values with different letters (a-b-c) were significantly different (Tukey, $p < 0.05$).

Table 2. Box-Behnken design with the observed responses of total sugar content (TSC) yield from (*Phoenix dactylifera L.*), using MA, UA and WB extractions.

Run	MAE					UAE					WBE					
	X_1	X_2	X_3	TSC (g/l)		X_1	X_2	X_3	TSC (g/l)		X_1	X_2	X_3	X_4	TSC (g/l)	
				Actual	Predicted				Actual	Predicted					Actual	Predicted
1	300	2	10	212.22 ±0.74	211.47	40	60	10	186.17 ±2.04	187.24	75	60	70	10	219.14 ±0.77	216.34
2	500	3	10	228.27 ±2.04	230.21	60	60	8	178.15 ±1.96	177.08	75	30	90	7	184.44 ±3.16	184.87
3	300	1	9	202.35 ±1.19	202.81	50	30	8	161.11 ±1.34	162.69	90	60	90	10	228.64 ±0.93	230.38
4	300	2	8	176.42 ±2.04	177.89	50	90	8	155.56 ±1.11	156.67	75	60	110	10	229.75 ±2.04	228.58
5	300	3	9	199.38 ±2.41	198.21	60	90	9	189.38 ±1.19	189.34	75	90	110	8.5	194.44 ±1.70	194.35
6	500	3	8	192.47 ±3.52	192.18	50	60	9	180.99 ±0.57	179.42	60	60	90	10	206.79 ±0.57	207.87
7	700	2	8	180.00 ±2.06	180.76	60	60	10	201.11 ±0.98	202.73	75	60	90	8.5	202.22 ±1.34	201.52
8	500	2	9	224.57 ±1.30	222.59	50	30	10	190.12 ±6.02	189.01	75	60	90	8.5	204.20 ±2.41	201.52
9	500	2	9	220.49 ±2.04	222.59	40	30	9	171.36 ±4.26	171.40	75	30	70	8.5	185.56 ±1.11	186.90
10	700	2	10	222.72 ±2.26	221.25	50	60	9	180.37 ±1.28	179.42	75	90	90	7	178.52 ±1.70	178.71
11	500	1	10	230.49 ±1.50	230.79	60	30	9	191.36 ±1.30	190.85	75	30	90	10	213.46 ±2.63	214.15
12	500	1	8	196.67 ±1.48	194.74	40	60	8	154.07 ±1.96	152.45	75	90	70	8.5	184.44 ±1.70	184.89
13	500	2	9	222.72 ±2.63	222.59	40	90	9	168.15 ±1.70	168.66	75	30	110	8.5	198.15 ±3.90	198.96
14	700	3	9	208.03 ±1.67	207.56	50	90	10	192.35 ±0.86	190.77	75	60	70	7	186.67 ±1.70	185.70
15	700	1	9	204.94 ±1.90	206.11	50	60	9	176.91 ±2.99	179.42	60	60	110	8.5	185.93 ±1.11	187.19
16											90	60	90	7	209.63 ±1.96	209.80
17											60	60	90	7	164.69 ±0.86	164.21
18											90	60	110	8.5	218.03 ±2.23	216.55

(Table 2) contd….

Run	MAE					UAE					WBE					
	X_1	X_2	X_3	TSC (g/l)		X_1	X_2	X_3	TSC (g/l)		X_1	X_2	X_3	X_4	TSC (g/l)	
				Actual	Predicted				Actual	Predicted					Actual	Predicted
19											90	30	90	8.5	209.26 ±2.89	208.66
20											75	60	110	7	194.32 ±1.50	194.98
21											75	90	90	10	213.21 ±1.67	213.67
22											60	60	70	8.5	169.38 ±1.71	171.74
23											90	90	90	8.5	200.74 ±0.37	201.28
24											60	30	90	8.5	173.21 ±0.93	170.54
25											90	60	70	8.5	210.86 ±1.67	210.48
26											75	60	90	8.5	198.15 ±2.31	201.52
27											60	90	90	8.5	172.84 ±1.07	171.30

Values are mean ± 95 % confidence interval.

the compounds of interest. However, a very long time can result a decrease in the efficiency of the extraction process. Therefore, the irradiation time must be appropriate. The extraction was carried out at different irradiation times, whereas other parameters were constant (500 W microwave power and 10:1 ml/g liquid/solid ratio). The yield of TSC significantly increased as the irradiation time increased from 30 s to 2 min. Then the recovery was maximized at 2 min and declined moderately with further increases in irradiation time (5 min). Since shorter extraction time is also favorable to reduce energy costs, the range 1-3 min was selected for the RSM study, while 2 min irradiation time was used for the last single factor trials dealing with a varying liquid/solid ratio.

For efficient extraction, the solvent volume must be sufficient to ensure complete immersion of materials, and an extraction solvent deficiency can lead to lower extraction yields (incomplete extraction) of ingredients [24], although redundant solvent may also lead to lower extraction yields and solvent waste [25]. Therefore, the liquid/solid ratio must be appropriate. The extraction was carried out at different liquid/solid ratios, whereas other extraction parameters were constant (500 W microwave power and 2 min irradiation time). The extraction yield of TSC significantly increased as the liquid/solid ratio increased within the range of 6:1-10:1 ml/g. However, the extraction yield did not change significantly when the liquid/solid ratio continued to increase (14:1 ml/g). Based on the statistical analysis, the range 8:1-10:1 ml/g was selected for the RSM optimization.

3.1.2. Optimization by RSM

Fifteen experiments were carried out according to the conditions indicated in Table **2**. Response values (TSC) are reported in the last column of this table. The combined effects of the microwave power, irradiation time, and liquid/solid ratio on the yield of TSC are shown in Fig. (**1**). The relationship between the response and the experimental variables can be illustrated graphically by plotting three-dimensional response surface plots (Figs. **1a-1c**). The vertical axes show the yield of TSC, and each of the two horizontal axes represents two of the three independent variables. In every plot, the factor not represented by the two horizontal axes was fixed at its 0 coded level. As can be seen in the figure and table, all the studied factors have a significant influence on the TSC, *i.e.* a significant increase in the recovery of total sugars with the elapse of irradiation time when microwave power was increased.

Fig. (**1a**) illustrates the interaction effect of microwave power and irradiation time, when the liquid/solid ratio was set at its 0 level (9:1 ml/g). The increase in microwave power and irradiation time increased the TSC up to a maximum of 224.57 g/l. However, a prolonged extraction time with the microwave power gave reduced TSC yield, which was also noticed during our preliminary study.

The effects of the interaction between microwave power and liquid/solid ratio on the TSC when the irradiation time was set at its 0 level (2 min) are presented in Fig. (**1b**). The results indicated that the yield increased with the increase of microwave power and liquid/solid ratio at the beginning of the extraction. The recovery reached its maximum (224.57g/l) at 500 W and 9:1 ml/g liquid/solid ratio during the MAE process. The increases in microwave power above 500 W led to decreases in the extraction recovery of TSC.

The effects of the interaction between irradiation time and liquid/solid ratio at 500 W (0 level) on the extraction yield of TSC are presented in Fig. (**1c**). TSC increased roughly from 192.47 to 230.49 g/l when the ratio increased from 8:1 to 10:1 ml/g and the irradiation time varied for 1-2 min; then, the decline was observed after 2 min.

The predicted yield (233.25 ± 3.59 g/l) obtained by the optimal conditions (power of 530 W, irradiation time of 1.99 min and liquid/solid ratio of 10:1 ml/g) was very close to the value predicted by the model (233.80 ± 1.90 g/l).

3.2. Ultrasound Assisted Extraction (UAE)

3.2.1. Effect of Independent Variables on the TSC Yield

The propagation of ultrasonic waves and cavitation effects are two key factors resulting in the enhancement of the

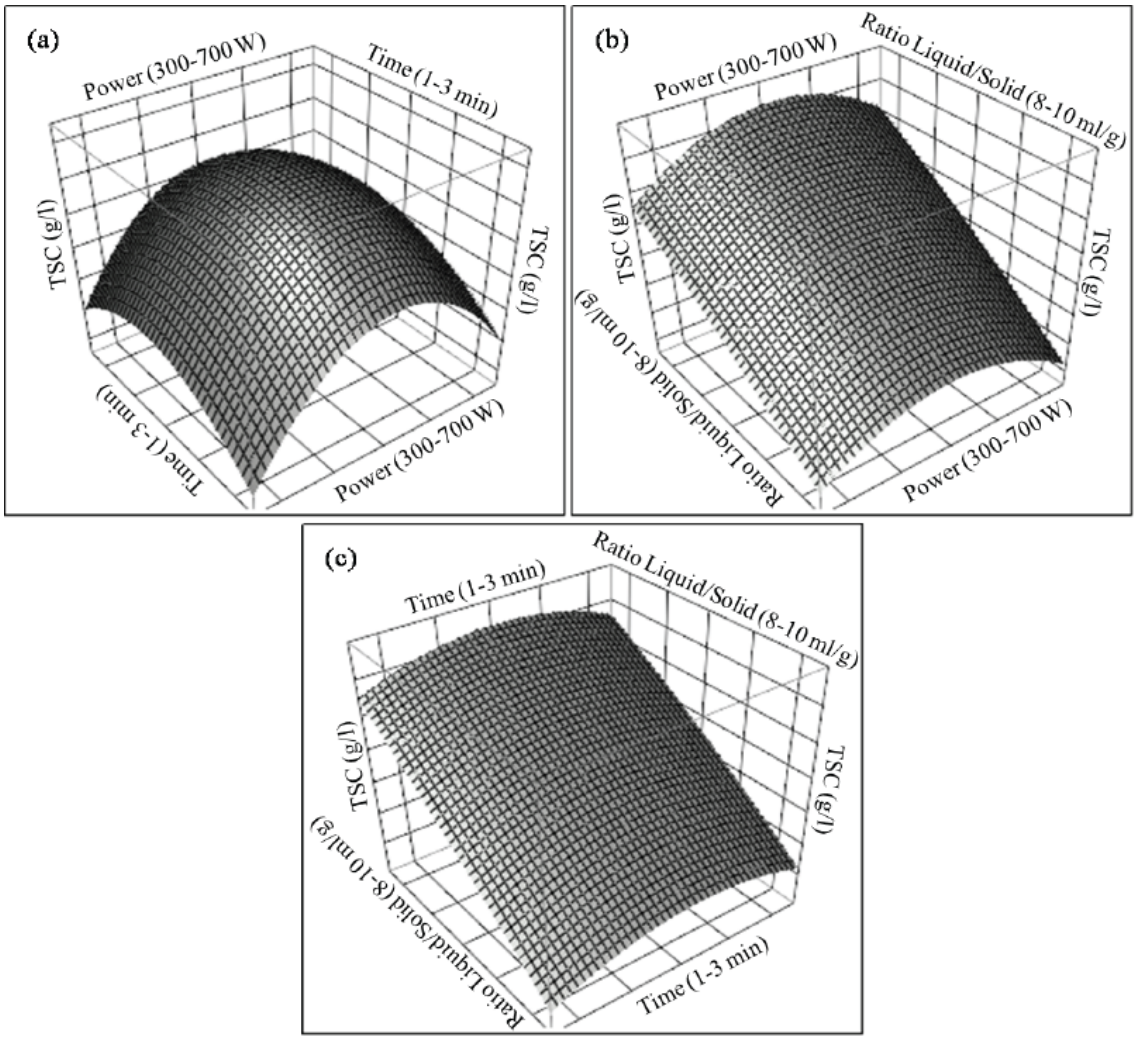

Fig. (1). Response surface analysis for the total sugar yield from *Phoenix dactylifera* L. by microwave-assisted extraction with respect to microwave power and irradiation time (**a**); microwave power and liquid/solid ratio (**b**) and irradiation time and liquid/solid ratio (**c**).

efficiency of the bioactive compounds extraction [26]. The cavitational effect, which increases mass transfer and results in close interaction between the solvent and plant tissues, is the principle of ultrasound-assisted extraction. Briefly, the implosion of cavitation bubbles generates macro-turbulence, high-velocity inter-particle collisions, and perturbation in micro-porous particles of the biomass, which accelerates the internal diffusion. Moreover, the collapse of cavitation bubbles in plant tissue surfaces produces micro-jets, leading to tissue disruption and good solvent penetration into the tissue matrix [27]. Usually, ultrasonic power is believed to be the driving force for the complete dispersion of solvent into the solid sample [28]. In this work, ultrasonic power was fixed to 42KHz. In the case of temperature, its increases can influence the membrane structure of the plant cells, making them less selective [29]. Additionally, at high extraction time, sugars can be degraded. Different temperatures were investigated (30-69°C) at a fixed ultrasonic treatment time of 30 min and 10:1 ml/g liquid/solid ratio. The TSC yield significantly increased when the ultrasonic temperature increased from 30 to 60°C. However, the extraction yields did not change significantly when the ultrasonic temperature continued to increase (69°C). Based on our observations, moderate ultrasonic temperature levels of 40, 50 and 60°C were selected as the lower, middle and upper levels, respectively, to apply in RSM optimization.

When the ultrasonic treatment time was at a low level (15 min), the yield of TSC increased to a certain value at 60 min with a fixed temperature of 60°C and 10:1ml/g liquid/solid ratio, after which, the yield decreased with increasing ultrasonic treatment time up to 90 min. The highest TSC yield was obtained at 60 min. Based on our results; the 30-60 min range was used for further experimentation using UAE for RSM optimization while 60 min was kept for the next single-factor trials.

A positive effect on TSC yield was recorded when the liquid/solid ratio was 10:1 ml/g. There was an increase in the yield with the increase of liquid/solid ratio from 6:1 to 10:1 ml/g. However, TSC was not significantly affected when the liquid/solid ratio continued to increase (14:1 ml/g). Based on the statistical analysis, the range 8:1-10:1 ml/g was finally selected for the RSM optimization.

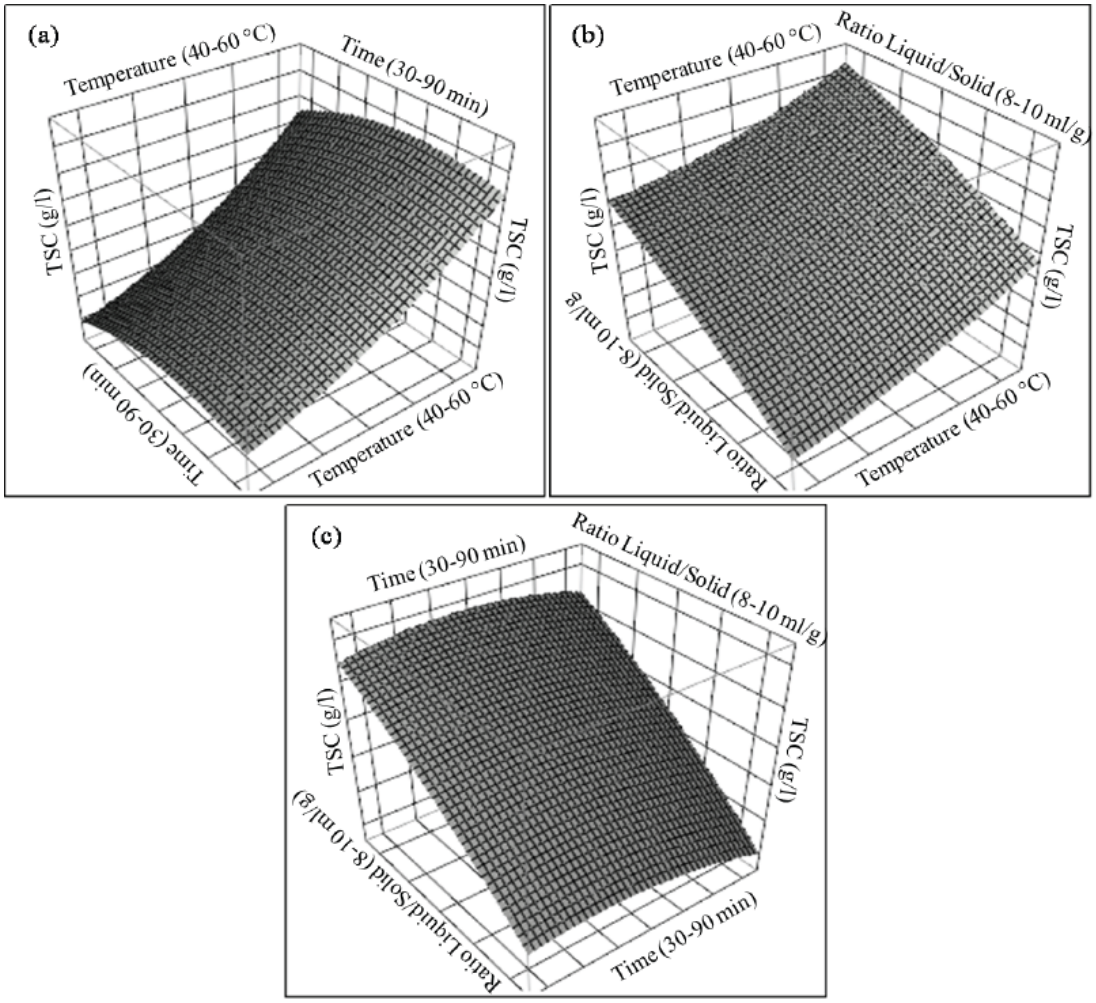

Fig. (2). Response surface analysis for the total sugar yield from *Phoenix dactylifera* L. by ultrasound-assisted extraction with respect to ultrasonic temperature and treatment time (**a**); ultrasonic temperature and liquid/solid ratio (**b**) and treatment time and liquid/solid ratio (**c**).

3.2.2. Optimization by RSM

In this optimization, we evaluated the effects of ultrasonic temperature, ultrasonic treatment time and liquid/solid ratio on TSC yield. The data obtained from 15 runs of experiments using UAE are shown in Table **2**. The three-dimensional response surface plots in Fig. (**2**) illustrate the relationship between the extraction yield of TSC and the experimental variables.

The effect of ultrasonic temperature and ultrasonic treatment time on the extraction yield of TSC at constant liquid/solid ratio (0 level) is shown in Fig. (**2a**). The extraction yield of TSC gradually increased with temperature and ultrasonic treatment time from 171.36to 191.36 g/land peaked at 60°C and 30 min. The extraction yield of TSC began to decrease beyond 60 min.

The appropriate liquid/solid ratio (10:1 ml/g) had positive effects on the extraction yield of TSC, as is shown in the response surface plots for the effect of liquid/solid ratio on the extraction yield (Fig. **2b** and **2c**) at constant ultrasonic treatment time and constant ultrasonic temperature, respectively. The extraction yields increased along with the increase in the liquid/solid ratio from 8:1 to 10:1 ml/g and ultrasonic temperature from 40 to 60°C to reach 201.11 g/l (Fig. **2b**). It was also observed that a simultaneous increase in the liquid/solid ratio from 8:1 to 10:1 ml/g and ultrasonic treatment time of 30-60 min increased the TSC yields from 178.15 to 201.11 g/l. After this increase, the TSC yields began to decrease beyond 60 min (Fig. **2c**).

The predicted yield (202.89 ± 5.80g/l) obtained by the optimal conditions (ultrasonic temperature 60°C, treatment time 68.10 min and liquid/solid ratio 10:1 ml/g) was very close to the value predicted by the model (202.04 ± 3.40 g/l).

3.3. Conventional Extraction (CE) or Water Bath Extraction (WBE)

3.3.1. Effect of Independent Variables on TSC Yield

It is known that high temperature can facilitate the release of sugars into the headspace by overcoming energy barriers that bind them to the matrix. Different temperatures were investigated from 40-90°C at a fixed treatment time of 30 min, 110 rpm shaking speed and 10:1 ml/g liquid/solid ratio. As indicated in this paragraph, most sugars were extracted efficiently at 90°C. Based on these results, the temperature range 60-90°C was selected for the RSM trials and

90°C was fixed for the next single-factor experiments on the influence of treatment time.

Treatment time is one of the most important parameters in the water bath extraction method. In this study, different extraction times were investigated, from 1-180 min, at a fixed temperature of 90°C, 110 rpm shaking speed and 10:1 ml/g liquid/solid ratio. An increase in efficiency was observed when the extraction time increased from 1 min to 60 min, and then it began to decrease beyond 60 min. Thus, the optimum equilibrium time was determined, and the range of 30-90 min was selected for the RSM trials, while 60 min was kept for the next single-factor trials.

Different liquid/solid ratios from 6:1 to 14:1 ml/g were selected to be compared for their effectiveness in TSC extraction, in terms of extraction quantity and repeatability at a fixed temperature of 90°C, 60 min extraction time and 110 rpm shaking speed. The 10:1 ml/g liquid/solid ratio presented a higher yield for the investigated compounds. Thus, based on these preliminary results, the 10:1 ml/g liquid/solid was selected for the extraction of total sugars in the subsequent experiments while the range 6:1-10:1 ml/g was selected for the RSM trials.

The addition of shaking speed as a fourth factor can increase the extraction yield. As a consequence, extraction efficiency is improved due to the favored diffusion of the analytes to the headspace. Different shaking speeds (30, 50, 70, 90 and 110 rpm) were used to investigate their influence on the conventional procedure at a fixed temperature 90°C, 60 min extraction time and liquid/solid ratio 10:1 ml/g. Increases in TSC yield were observed with the increasing of shaking speed from 30 to 110 rpm. Thus, higher level resulted in higher extraction efficiency. Therefore, 110 rpm shaking speed was chosen as the best condition in this regard while the range 70 to 110 rpm was selected for the RSM trials.

3.3.2. Optimization by RSM

The experimental design and corresponding response data for the TSC from *Phoenix dactylifera* L. for the conventional extraction are presented in Table **2**, with twenty seven experiments. The effect of temperature, treatment time, liquid/solid ratio and shaking speed on the extraction yield of TSC was investigated, and the results are shown in Fig. (**3**). The three-dimensional response surface plots in this figure illustrate the relationship between the TSC extraction yield and the experimental variables. The plots were generated by plotting the response using the z-axis against two independent variables (X_1 and X_2) while keeping the other two independent variables (X_3 and X_4) at their zero level. These results indicate that all tested parameters have a great impact on TSC extraction yield.

The profiles obtained for the effect of temperature and treatment time on TSC extraction yield at 0 levels, fixed shaking speed and liquid/solid ratio are shown in Fig. (**3a**). The extraction yield of TSC gradually increased with temperature and treatment time and peaked at approximately 90°C and 30 min to reach 209.26 g/l. Further increase in treatment time enhanced the degradation of the investigated compounds, which resulted in the decrease of the TSC to 200.74 g/l.

With respect to the interaction between temperature and shaking speed when treatment time and liquid/solid ratio were set at their 0 levels, presented in Fig. (**2b**), by increasing the temperature and the shaking speed from 60 to 90°C and 70 to 110 rpm, respectively, the TSC increased from 169.38 to 218.03 g/l and peaked at approximately 90°C and 110 rpm shaking speed. Fig. (**1d**). illustrates the interaction effect of extraction time and shaking speed, when the temperature and liquid/solid ratio were set at their 0 levels (75°C and 8.5:1 ml/g); the increase in the extraction time and shaking speed increased the TSC up to a maximum of 204.20 g/l. However, a prolonged extraction time reduced it; this result was also noticed during our preliminary study.

The response surface plots for the effect of liquid/solid ratio on extraction yield (Figs. **3c-3e-3f**) at constant temperature, treatment time and shaking speed show that an appropriate extraction liquid/solid ratio (10:1 ml/g) had a positive effect on the extraction yield of TSC (Fig. **3c**), when the 3D response surface plot was developed for the recovery of TSC with varying temperature and liquid/solid ratio. It can be observed that a maximum recovery of TSC (228.64 g/l) was achieved with a temperature of90°C at a liquid/solid ratio of 10:1 ml/g. In the latter case, the extraction was increased when the treatment time was lower than 60 min and decreased when it exceeded 60 min. The interactive terms, in the model, are not significant. Fig. (**3e**) shows that the TSC could be maximized to 213.46 g/l at about 30 min and 10:1 ml/g liquid/solid ratio over a range of the other operational factors (temperature and shaking speed). In fact, in water bath extraction, shaking speed is one of the key variables affecting the release of sugars from different matrices. The increase in the shaking speed accelerated sugar extraction. Fig. (**3f**) shows that with an increase in shaking speed from 70 to 110 rpm and liquid/solid ratio from 7:1 to 10:1 (ml/g), the extraction yield of TSC increased gradually from 186.67 to 229.75 g/l.

From the model, the optimal conditions of CE were: temperature 90°C, extraction time 55.34 min, 110 rpm shaking speed and liquid/solid ratio 10:1 ml/g with a predicted yield of 233.54 ± 5.41g/l. CE was carried out under these conditions giving a real recovery of 234.38 ± 2.43 g/l.

3.4. Verification of the Predictive Models

Based on the above findings, to validate the predictability of the established models, the optimized parameters were tested. The mean values 233.25 ± 3.59 g/l, 202.89 ± 5.80g/l and 233.54 ± 5,41g/l were found in the actual experiments for MAE, UAE and CE, respectively, which were in close agreement with the predicted values (233.80 ± 1.90 g/l, 202.04 ± 3.40 g/land 234.38 ± 2.43 g/l) using the respective model Eqs. (3), (4) and (5). The strong correlation between these results confirmed that the response models were adequate in predicting the optimized conditions.

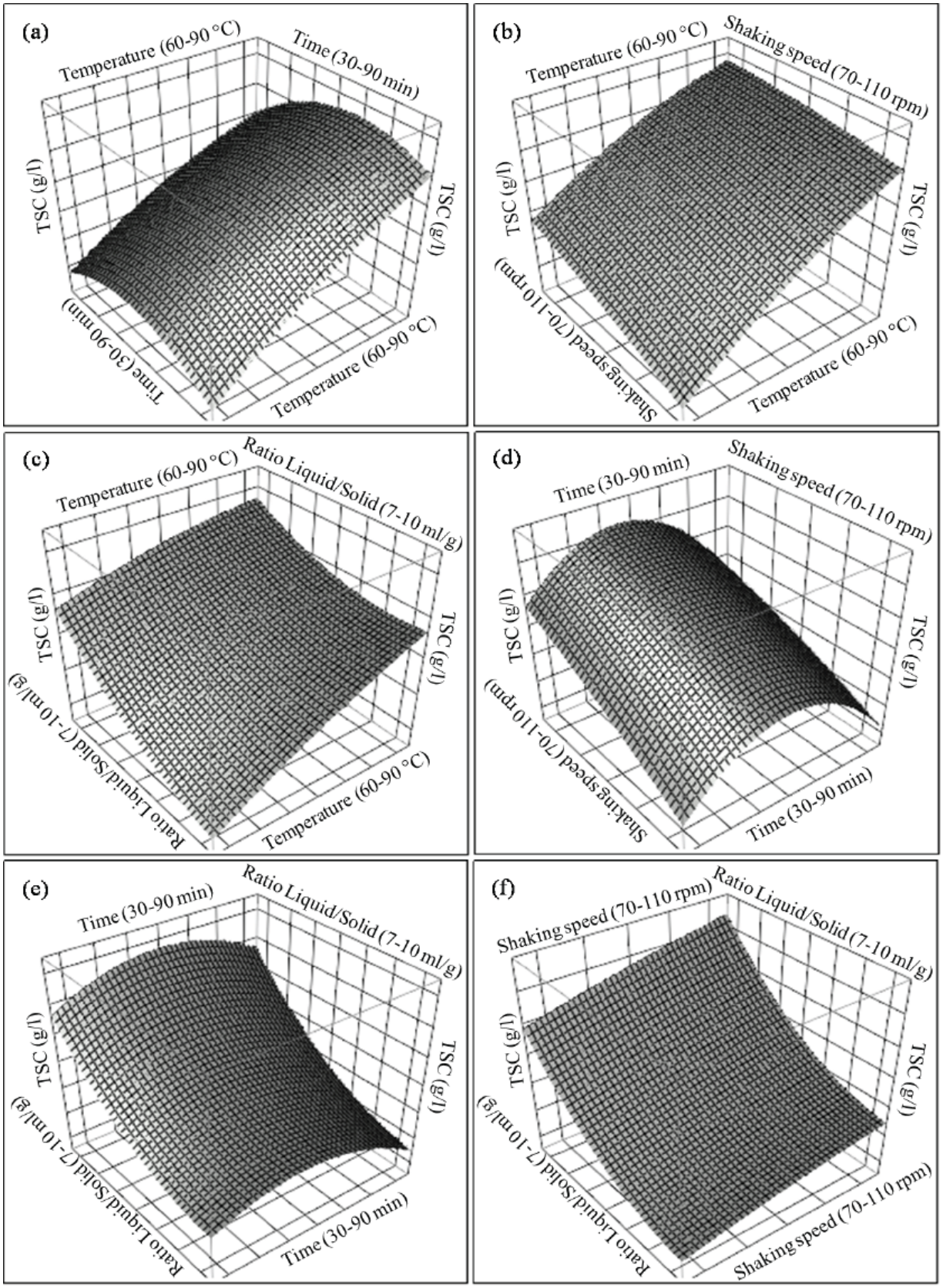

Fig. (3). Response surface analysis for the total sugar yield from *Phoenix dactylifera* L. by water bath extraction with respect to temperature and extraction time (**a**); temperature and shaking level (**b**); temperature and liquid/solid ratio (**c**); extraction time and shaking level (**d**); extraction time and liquid/solid ratio (**e**); shaking level and liquid/solid ratio (**f**).

3.5. Fitting the Response Surface Models

The empirical relationship between the extraction yield of TSC and the independent variables for MAE, UAE and CE, respectively, expressed by the quadratic model, are given in Eqs. (3), (4) and (5).

$$Y(TSC) = 222.59 + 3.16X_1 - 0.79X_2 + 18.52X_3 + 1.51X_1X_2 + 1.73X_1X_3 + 0.49X_2X_3 - 16.53X_1^2 - 2.39X_2^2 - 8.23X_3^2 \quad (3)$$

$$Y(TSC) = 179.42 + 10.03X_1 - 1.06X_2 + 15.11X_3 + 0.30X_1X_2 - 2.28X_1X_3 + 1.94X_2X_3 + 2.87X_1^2 - 2.23X_2^2 - 2.41X_3^2 \quad (4)$$

$$Y(TSC) = 201.52 + 17.03X_1 - 1.66X_2 + 5.38X_3 + 16.06X_4 - 2.04X_1X_2 - 2.35X_1X_3 - 0.65X_2X_3 - 5.77X_1X_4 + 1.42X_2X_4 + 0.74X_3X_4 - 4.18X_1^2 - 9.40X_2^2 - 0.85X_3^2 + 5.73X_4^2 \quad (5)$$

where Y is the extraction yield of TSC and X_1, X_2, X_3 and X_4 are the uncoded variables.

In general, the exploration and optimization of a fitted response surface may produce poor or misleading results unless the model exhibits a good fit, which makes it essential to verify the model's adequacy [30-32]. Table **3** shows the results of the second-order response surface in the form of analysis of variance (ANOVA). The significance of each coefficient was determined by the *F*-test and *p*-value, which are listed in Table **3**. The corresponding variables would be more significant if the absolute *F*-value becomes greater and the *p*-value becomes smaller [33].

It can be seen that the independent variables, microwave power (X_1), irradiation time (X_2) and liquid/solid ratio (X_3) for MAE, ultrasonic temperature (X_1), ultrasonic treatment time (X_2) and liquid/solid ratio (X_3) for UAE and for WBE, temperature (X_1), treatment time (X_2), liquid/solid ratio (X_3) and shaking speed (X_4), with the largest effect on the response ($p<0.0001$) were the linear terms. The results suggest that the change in these variables have highly significant effects on the yield of TSC ($p<0.0001$), when MAE, UAE and CE are used to extract sugars from *Phoenix dactylifera* L. The non-significant value of the lack of fit (more than 0.05) showed that the quadratic models were valid for the present study.

The Fisher's *F*-values (4040.3), (2751.5) and (8151.5) with a very low probability value [($p>F$) < 0.0001] implied that the models were highly significant. The goodness of fit of the models were examined by determination of coefficients (R_1^2=0.9940), (R_2^2=0.9910) and (R_3^2=0.9930) for MAE, UAE and CE, respectively, which implied that the samples variation (99.40,99.10 and99.30%) were statistically significant and only 0.06 %, 0.09 % and 0.07 %, respectively, of the total variance could not be explained by the model. The adjusted determination coefficients (Adj. R_1^2=0.9831) (Adj. R_2^2=0.9747) (Adj. R_3^2=0.9849) and the predicted determination coefficients (Pred. R_1^2=0.8881) (Pred. R_2^2= 0.9448) (Pred. R_3^2=0.9035) were also satisfactory to confirm the significance of the models.

3.6. Comparative Study of the Three Extraction Processes (MAE, UAE and CE)

Date syrups were made from date juices extracted by three methods. The two alternative extraction technologies, MAE and UAE, were compared with each other and with CE considering the TSC yield of the extracts. A set of samples were extracted under the optimum conditions, and the predicted results fitted well with the experimental results. The following results were obtained: 233.80 ± 1.90g/l for MAE, 202.04 ± 3.40g/l for UAE and 234.38 ± 2.43 g/l for CE.

The selection of an extraction method would mainly depend on the advantages and disadvantages of the processes such as extraction yield, complexity, production cost, environmental friendliness and safety [34]. The results indicated that MAE showed a similar extraction capacity for the TSC as compared to CE; however, the extracted TSC by UAE was significantly lower than that of MAE and CE. This result is not in agreement with that reported by Ganbi (2012) [35], who found the highest total sugar content (88.94%) in the syrup extracted by ultrasonic procedure. The concentration using MAE greatly reduced the processing time; the shortest processing time (1.99 min) occurred at the power of 530 W. However, conventional extraction took the same time as ultrasound-assisted extraction, which was about 60 min. Microwave-assisted extraction was the most efficient method for extracting sugars based on the lower extraction time.

3.7. Identification of Sugars from Date Syrups by HPLC

Carbohydrates are the major chemical constituents of dates, including mainly reducing sugars such as glucose and fructose, and also non-reducing sugars such as sucrose, and small amounts of polysaccharides such as cellulose and starch [36]. In the present study, reducing sugars (glucose and fructose) and sucrose were identified in the date syrups obtained by MAE, UAE and CE methods (39.89, 16.64 and 23.25 %; 00.00, 46.94 and 53.06% and 49.22, 20.67 and 28.55 %, respectively). Concerning these two reducing sugars, all syrups contain more fructose than glucose, which is in agreement with previous studies [37]. High-fructose syrup is largely used as a sweetener in the food and pharmaceutical industries, as well as to attain crystalline fructose. Moreover, compared to sucrose syrup, it has more desirable functional properties, such as high osmotic pressure, high solubility, and itprevents the crystallization of sugar in food products [38]. Glucose and fructose were the only monomers of the reducing sugars present in date syrup, and they are the most important sources of energy with high digestibility [39]. These two sugars are the product of the enzymatic reaction of invertase on the disaccharide (sucrose). This enzyme hydrolyses sucrose into glucose and fructose completely in soft date cultivars, and partially in semi-dry and dry date cultivars [35]. Indeed, the increase in invertase activity is the main factor for the reducing sugar increase [40], and a direct correlation was found between the reducing sugar content of dates and the invertase activities [38]. As we can see, and

Table 3. Analysis of variance (ANOVA) for the experimental results of MA, UA and WB extractions.

MAE				UAE				WBE			
	Sum of Squares	**F-value**	**P-value**		**Sum of Squares**	**F-value**	**P-value**		**Sum of Squares**	***F*-value**	***p*-value**
Model	4040.3	91.4	< 0.0001	Model	2751.5	60.89	< 0.0001	Model	8151.5	122	< 0.0001
X_1-Power	3.16	4.04	0.0099	X_1-Tem-perature	10.03	12.66	< 0.0001	X_1- Tem-perature	17.03	26.9	< 0.0001
X_2-Time	-0.79	-1.00	0.3613	X_2-Time	-1.06	-1.43	0.2368	X_2-Time	-1.66	-2.63	0.0221
X_3-Ratio	18.52	23.63	< 0.0001	X_3-Ratio	15.11	19.07	< 0.0001	X_3-Shaking	5.38	8.53	< 0.0001
X_1X_2	1.51	1.36	0.2305	X_1X_2	0.31	0.28	0.79	X_4-Ratio	16.06	25.5	< 0.0001
X_1X_3	1.72	1.56	0.1795	X_1X_3	-2.28	-2.04	0.10	X_1X_2	-2.04	-1.86	0.0869
X_2X_3	0.49	0.45	0.6744	X_2X_3	1.94	1.74	0.14	X_1X_3	-2.35	-2.15	0.0529
X_1^2	-16.53	-14.33	< 0.0001	X_1^2	2.87	2.46	0.06	X_1X_4	-5.77	-0.59	0.5641
X_2^2	-2.39	-2.07	0.0928	X_2^2	-2.23	-1.91	0.11	X_2X_3	-0.65	-5.28	0.0002
X_3^2	-8.23	-7.13	0.0008	X_3^2	-2.41	-2.07	0.09	X_2X_4	1.42	1.30	0.2183
Residual	24.56			Residual	25.11			X_3X_4	0.74	0.68	0.5106
Lack of Fit	91.40	1.30	0.46	Lack of Fit	60.89	1.07	0.52	X_1^2	-4.18	-4.42	0.0008
Pure Error	8.32			Pure Error	9.64			X_2^2	-9.40	-9.93	< 0.0001
R^2	0.99			R^2	0.99			X_3^2	-0.85	-0.90	0.0008
R^2_{adj}	0.98			R^2_{adj}	0.97			X_4^2	5.73	6.05	< 0.0001
Adeq pre				Adeq pre				Residual	57.29		
RMSE	2.22			RMSE	2.24			Lack of Fit	121.95	0.40	0.87
Cor Total	24.56			Cor Total	25.11			Pure Error	19.03		
								R^2	0.99		
								R^2_{adj}	0.98		
								Adeq pre			
								RMSE	2.19		
								Cor Total	8208.8		

except for the syrup obtained by UAE, the major sugar is the non-reducing one (sucrose) in both MAE and CE syrups; theircontent represents more than half (60.11 %) of the reducing sugars in the syrup obtained by MAE and about half (50.78 %) of that prepared by CE or WBE. Indeed, dry dates contain relatively more sucrose than reducing sugars [41], which confers a very pleasant taste to the fruit [42]. It is also worth highlighting the absence of sucrose in the syrup obtained by UAE, which could be explained by the increases of invertase activity, during the ultrasound treatment. It has been reported that sucrose hydrolysis by invertase is accelerated by ultrasound irradiation [43]. On the other hand, the reducing sugar release, in cassava chip slurry, was as high as 180% of the control samples and the slurry samples with enzyme addition during sonication resulted in greater release than samples with enzyme addition after sonication [44].

CONCLUSION

In this study, novel extraction techniques (MAE and UAE) were compared with a conventional one in the extraction of TSC from *Phoenix dactylifera* L. and RSM was suc-

cessfully applied to optimize the three extraction methods. Microwave power, ultrasonic temperature, water bath temperature, extraction time, liquid/solid ratio and shaking speed played significant roles in the extraction of the investigated compounds. We were able to extract higher yields of the constituent from *Phoenix dactylifera* L., while using microwave-assisted extraction, which simultaneously reduced the extraction time considerably compared with two other methods that take along extraction time. However, if we consider the nature of the sugars extracted by these three methods, the ultrasound technique is the one that gives the best yields of reducing sugars and especially fructose, which is known for its desirable functional properties.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Aspé E, Fernández K. The effect of different extraction techniques on extraction yield, total phenolic, and anti-radical capacity of extracts from *Pinus radiata* Bark. Ind Crops Prod 2011; 34: 838-44.

[2] Dahmoune F, Boulekbache L, Moussi K, Aoun O, Spigno G, Madani K. Valorization of Citrus limon residues for the recovery of antioxidants: Evaluation and optimization of microwave and ultrasound application to solvent extraction. Ind Crops Prod 2013; 50: 77-87.

[3] Aybastıer Ö, Işık E, Şahin S, Demir C. Optimization of ultrasonic-assisted extraction of antioxidant compounds from blackberry leaves using response surface methodology. Ind Crops Prod 2013; 44: 558-65.

[4] Muniz-Marquez DB, Martinez-Avila GC, Wong-Paz JE, Belmares-Cerda R, Rodriguez-Herrera R, Aguilar CN. Ultrasound-assisted extraction of phenolic compounds from Laurus nobilis L. and their antioxidant activity. Ultrason Sonochem 2013; 20: 1149-54.

[5] Chandrasekaran M, Bahkali AH. Valorization of date palm (*Phoenix dactylifera*) fruit processing by-products and wastes using bioprocess technology - Review. Saudi J Biol Sci 2013; 20: 105-20.

[6] Abbès F, Kchaou W, Blecker C, Ongena M, Lognay G, Attia H, Besbes S. Effect of processing conditions on phenolic compounds and antioxidant properties of date syrup. Ind Crops Prod 2013; 44: 634-42.

[7] Besbes S, Drira L, Blecker C, Deroanne C, Attia H. Adding value to hard date (*Phoenix dactylifera* L.): Compositional, functional and sensory characteristics of date jam. Food Chem 2009; 112: 406-11.

[8] Abbès F, Bouaziz MA, Blecker C, Masmoudi M, Attia H, Besbes S. Date syrup: Effect of hydrolytic enzymes (pectinase/cellulase) on physico-chemical characteristics, sensory and functional properties. LWT - Food Sci Technol 2011; 44: 1827-34.

[9] Boudries H, Kefalas P, Horneromendez D. Carotenoid composition of Algerian date varieties (*Phoenix dactylifera*) at different edible maturation stages. Food Chem 2007; 101: 1372-7.

[10] Baliga MS, Baliga BRV, Kandathil SM, Bhat HP, Vayalil PK. A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). Food Res Int 2011; 44: 1812-22.

[11] Al-Hooti SN, Sidhu JS, Al-Saqer JM, Al-Othman A. Chemical composition and quality of date syrup as affected by pectinase/cellulase enzyme treatment. Food Chem 2002; 79: 215-20.

[12] Gad A, Kholif A, Sayed A. Combination of date palm syrup and skim milk. Am J Food Technol 2010; 5: 250-59.

[13] Manaa S, Younsi M, Moummi N. Study of methods for drying dates; Review the traditional drying methods in the region of Touat Wilaya of Adrar-Algeria. Energy Procedia 2013; 36: 521-4.

[14] Benmeddour Z, Mehinagic E, Meurlay DL, Louaileche H. Phenolic composition and antioxidant capacities of ten Algerian date (*Phoenix dactylifera* L.) cultivars: A comparative study. J Funct Foods 2013; 5: 346-54.

[15] Chibane H, Benamara S, Noui Y, Djouab A. Some physicochemical and morphological characterizations of three varieties of Algerian common dates. Eur J Sci Res 2007; 18: 134-40.

[16] El Ogaïdi H. Dates and confectionery product. FAO Rome 1987; 1-25.

[17] Munier P. Le palmier-dattier, Techniques agricoles et productions tropicales. Maisonneuve & Larose: Paris 1973.

[18] Guérin C. Première biozonation du Pléistocène européen, principal résultat biostratigraphique de l'étude des Rhinocerotidae (Mammalia, Perissodactyla) du Miocène terminal au Pléistocène supérieur d'Europe occidentale. Geobios 1982; 15: 593-8.

[19] Hashemi SMB, Khaneghah AM, Saraiva JA, Jambrak AR, Barba FJ, Mota MJ. Effect of ultrasound on lactic acid production by *Lactobacillus* strains in date (*Phoenix dactylifera var. Kabkab*) syrup. Appl Microbiol Biotechnol 2018; 102:2635-44.

[20] Multon J. interet des additifs pour ameliorer la conservation des aliments, Additifs et auxiliaires de fabrication dans les industries agro-alimentaires/coordonnateur, JL Multon; preface, F. Lepatre 1984.

[21] Dreywood R. Qualitative test for carbohydrate material. Ind Eng Chem Anal Ed 1946; 18: 499.

[22] Hanson R, Phillips J. Chemical composition. Manual of methods for general bacteriology. American Society for Microbiology: Washington DC 1981; pp. 328-64.

[23] Sahin S, Samli R. Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Ultrason Sonochem 2013; 20: 595-602.

[24] Sun Y, Wang W. Ultrasonic extraction of ferulic acid from Ligusticum chuanxiong. J Chin Inst Chem Eng 2008; 39: 653-6.

[25] Zou Y, Chen X, Yang W, Liu S. Response surface methodology for optimization of the ultrasonic extraction of polysaccharides from *Codonopsis pilosula* Nannf. var. *modesta* LT Shen. Carbohydr Polym 2011; 84: 503-8.

[26] Ji J, Lu X, Cai M, Xu Z. Improvement of leaching process of geniposide with ultrasound. Ultrason sonochem 2006; 13: 455-62.

[27] Vilkhu K, Mawson R, Simons L, Bates D. Applications and opportunities for ultrasound assisted extraction in the food industry—A review. Innov Food Sci Emerg Technol 2008; 9: 161-9.

[28] Han D, Zhu T, Row KH. Ultrasonic extraction of phenolic compounds from *Laminaria japonica* Aresch using ionic liquid as extraction solvent. Bull Korean Chem Soc 2011; 32: 2212-6.

[29] Fernandez D, Goodwin A, Lemmon EW, Sengers JL, Williams R. A formulation for the static permittivity of water and steam at temperatures from 238 K to 873 K at pressures up to 1200 MPa, including derivatives and Debye-Hückel coefficients. J Phys Chem Ref Data 1997; 26: 1125-66.

[30] Bimakr M, Rahman RA, Ganjloo A, Taip FS, Salleh LM, Sarker MZI. Optimization of supercritical carbon dioxide extraction of bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves by using response surface methodology. Food Bioproc Tech 2012; 5: 912-20.

[31] Liyana-Pathirana Cand Shahidi F. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem 2005; 93: 47-56.

[32] Scavroni J, Boaro CSF, MarquesMOM, Ferreira LC. Yield and composition of the essential oil of *Mentha piperita* L.(Lamiaceae) grown with biosolid. Braz J Plant Physiol 2005; 17: 345-52.

[33] Atkinson A, Donev A. Optimum experimental designs. Clarendon Press: UK 1992.

[34] Li H, Deng Z, Wu T, Liu R, Loewen S, Tsao R. Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. Food Chem 2012; 130: 928-36.

[35] Ganbi HHA. Production of nutritious high quality date (*Phoenix dactylifera*) fruits syrup (dibs) by using some novel technological approaches. J Appl Sci Res 2012; 8: 1524-38.

[36] Ashraf Z, Hamidi-Esfahani Z. Date and date processing: A review. Food RevInt2011; 27: 101-33.

[37] Al-Eid SM. Chromatographic separation of fructose from date syrup. Int J Food Sci Nutr 2006; 57: 83-96.

[38] Chaira N, Smaali I, Besbes S, Mrabet A, Lachiheb B, Ferchichi A. Production of fructose rich syrups using invertase from date palm fruits. J Food Biochem2011; 35: 1576-82.

[39] Al-Farsi M, Alasalvar C, Al-Abid M, Al-Shoaily K, Al-Amry M, Al-Rawahy F. Compositional and functional characteristics of dates, syrups, and their by-products. Food Chem 2007; 104: 943-7.

[40] Hasegawa S, Smolensky DC. Date invertase: Properties and activity associated with maturation and quality. J Agr Food Chem 1970; 18: 902-4.

[41] Eltayeb EA, Al-hasni AS, Farooq SA. Changes in soluble sugar content during the development of fruits in some varieties of omani date palm (*Phoenix dactylifera*). Pak J Biol Sci 1999; 2: 255-8.

[42] Mrabet A, Ferchichi A, Chaira N, Mohamed BS. Physico-chemical characteristics and total quality of date palm varieties grown in the Southern of Tunisia. Pak J Biol Sci 2008; 11: 1003-8.

[43] Kwiatkowska B, Bennett J, Akunna J, Walker GM, Bremner DH. Stimulation of bioprocesses by ultrasound. Biotechnol Adv 2011; 29: 768-80.

[44] Nitayavardhana S, Rakshit SK, Grewell D. Ultrasound pretreatment of cassava chip slurry to enhance sugar release for subsequent ethanol production. Biotechnol Bioengin 2008; 101: 487-96.

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ORIGINAL ARTICLE

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Dairy dessert processing: Effect of sugar substitution by date syrup and powder on its quality characteristics

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Abstract

Second-grade dates can be valorized by its uses in the production of nutritive foods with health benefits. Date syrup (DS), date powder (DP), and their different concentrations, using D-optimal mixture design, were used for the formulation of dairy desserts: syrup dairy dessert (SDD) and mixture dairy dessert (MDD). The optimized mixture proportions of the best dairy dessert formulation, containing date syrup (14%) and powder (2%) as a sugar substitute (0%), were determined to achieve the desired sensory for the effective formulation process. Significant differences in water, protein, fat, and sugar contents were found between SDD, MDD, and commercial dairy dessert (CDD) used as standard, respectively. SDD exhibited the highest total phenolic content (0.65 ± 0.04 mg GAE/g), DPPH inhibition (4.00 ± 0.82%), and reducing power (2.75 ± 0.10%) followed by MDD and CDD. The absence of germs indicated a high microbiological quality of dairy desserts. Rheological measurements revealed that all samples exhibited a weak gel structure and shear thinning behavior. Desserts supplemented with dates led to desserts with an homogeneous structure.

Practical applications

The evolution of technological processes, conservation techniques, and distribution has led to the development of a wide range of "milks for consumption". The success of these fresh dairy desserts is explained by their beneficial effect on health and organoleptic properties that are appreciated by the consumers. The most delicate in the production of dairy dessert is the development of a "formulation" with the choice of ingredients, and their respective proportion; the amount of sugar, aroma, taste, and color. *Phoenix dactylifera* L. have a vital therapeutic properties, it represents an inexhaustible source of bioactive compounds such as polyphenols. So the objective of the present study is to formulate new dairy desserts with date syrup and powder (by-products from common date fruits) as a sweetener-ingredient in order to substitute sugars.

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1 | INTRODUCTION

Date palm fruit (*Phoenix dactylifera* L.) is an endemic functional food, with great nutritional and economic importance due to its phytochemical compositions (Al Alawi, Alhamdani, Hoheisel, & Baqi, 2020). Dates are considered as the main crop produced in Algeria; with 1,058,559 tons harvested in 2017, it ranks the third worldwide (FAOSTAT, 2018). In fact, they are rich in nutrients (Nadeem et al., 2018) and provide a good source of rapid energy due to their carbohydrate content (70–80%). Moreover, date fruit contain fats (0.20–1.50%), proteins (0.30–2.50%), minerals (0.10–9.16 mg/100 g dry weight), vitamins (C, B_1, B_2, B_3 and A), and dietary fibers (6.40–11.50%), with no starch (Al-Shahib & Marshall, 2003).

The lost dates, commonly named "date by-product," are not consumed by humans because of their inadequate texture (too soft or too hard), contamination with fungus as well as infestation by insects or simply due to their low quality (Besbes, Drira, Blecker, Deroanne, & Attia, 2009). These by-products can be processed into different products including date syrup (Hashim, Omer, & Fathelrahman, 2016). Date syrup called dibs or molasses is probably the most common derived date product. It can be directly consumed or used as a natural sweetener and flavoring for human consumption in some food formulations such as ice cream, confectionery, bakery products, sesame paste/date syrup blends, jam, and butter (Abbès et al., 2013), as well as milk beverage (Keshtkaran, Mohammadifar, Asadi, Nejad, & Balaghi, 2013), yogurt (Milani, Koocheki, & Golimovahhed, 2011), and dairy dessert (Jridi et al., 2015). In addition, currently, the global health market has been invaded by functional foods (Silva, Silva, Costa-Lima, Carneiro, & Conte-Junior, 2017). In this context, these products provide a health benefit beyond the traditional nutrients it contains, so they are used to prevent certain diseases (Vayalil, 2012).

Consumers show an increasing demand for healthier food products, desirably free of chemical additives (Sayas-Barberá, Martín-Sánchez, Cherif, Ben-Abda, & Pérez-Álvarez, 2020). The incorporation of date syrup and powder in the formulation of dairy desserts could be an alternative for the valorization of industrial by-products as well as by the manufacture of health and functional food products with high added values. Jridi et al. (2015) aimed to produce syrups and powders from three Tunisian date varieties (Deglet Nour, Kentichi, and Allig) served as additives to prepare dairy dessert formulations.

Currently, the food market forces the industries concerned to formulate new products that meet the requirements of the consumer, from the functional properties, nutritional, and therapeutic point of view. Recent studies have been conducted dealing with the effect of alternative sweeteners to sucrose on rheological behavior, sensory and physicochemical properties, and identifying several beneficial attributes of dairy desserts (Gurditta, Patel, & Arora, 2019; Jahromi & Niakousari, 2018; Prasad, Khamrui, Mandal, & Badola, 2018). However, the available studies only evaluate the impact of this substitution on the characteristics of the products. In this sense, the manufacturing process of dairy desserts is not studied in an optimization point of view.

In this study, the objective is to optimize the formulation of new dairy desserts with date syrup and powder (by-products from common date fruits) as a sweetener-ingredient, using the D-optimal mixture design, in order to substitute sugars. As the formulation of new food products influence the final product quality, particularly texture, aroma, and flavor; we have investigated the possible influence of the incorporation on sensory, physicochemical, phytochemical, microbiological, rheological, and microstructural characteristics of the dairy desserts according to the consumer preferences (syrup dairy dessert [SDD] and optimal formulation of mixture dairy dessert [MDD]) and the results are compared with a commercial dairy dessert (CDD).

2 | MATERIAL AND METHODS

2.1 | Material

Dates used in this study (Degla–Beida) correspond to a dry nature and hard texture from South-East Algeria. This fruit is abundant on the national territory; with a low market value; long shelf life; and nutritional quality (high sugar content). The sample was purchased from a local market in Bejaia city (Algeria). All solvents and reagents were purchased from Sigma-Aldrich.

2.2 | Preparation of samples

2.2.1 | Date powder

Dates were washed, pitted, and dried at 40°C until up to a constant weight. Then, they were grounded into a fine powder, which was passed through a standard sieve and only the fraction with particle size ≤125 μm was used. Finally, the powder was stored in tight bottles in the darkness.

2.2.2 | Date syrup

Date syrup was prepared by mixing 10 g of date powder with 100 ml of water as described by Djaoud et al. (2019). The mixture was treated under microwave irradiations (MAXMOS23S, Maxipower, China) at a power input of 500 W with permanent irradiation and cooling time of 2 min. After that, the extracts were filtered under vacuum and, posteriorly, centrifuged (NÜVE mod.NF200, Ankara, Turkey) at 2,258 × g for 15 min. The obtained juices were concentrated at 70°C until having syrup at 80°Brix.

2.2.3 | Dairy desserts

Dairy desserts were formulated as described by Hamza-Chaffai (1990). The whole of work was carried out based on milk, corn starch, carrageenan, date syrup, and date powder.

2.3 | Experimental design

Mixture design was practical to establish the optimal concentrations for greatest formulation of the dairy desserts generated by the statistical software package JMP (10.0.0 version, SAS Institute, USA). In this study, a three-factor, two-level D-optimal mixture design was employed to construct polynomial models for the optimization process. The selected factors were: X_1—Date syrup, X_2—Date powder, and X_3—Sugar, whereas the responses were the following descriptors: Y_1—Texture, Y_2—Hardness, Y_3—Granularity, Y_4—Sweetness, Y_5—Color, Y_6—Odor, and Y_7—Savor. Table 1 shows the coded variables and observed responses for the D-optimal mixture design of the six (n = 6) formulations suggested by the Design-Expert software prepared with different concentrations of date syrup, date powder, and sugar, respectively: MDD_1 (0.125–0.875–0), MDD_2 (0.125–0.125–0.75), MDD_3 (0.125–0.125–0.75), MDD_4 (0.125–0.875–0), MDD_5 (0.875–0.125–0), and MDD_6 (0.875–0.125–0). In addition, syrup dairy dessert SDD (1–0–0) and powder dairy dessert PDD (0–1–0) were evaluated.

2.4 | Sensory evaluation

As mentioned by Wang, Liu, Jing, Fan, and Cai (2019), 10 panelists (n = 10; including five females and five males, aging from 27 to 50 years) familiar with sensory evaluation techniques and regular consumers of dairy desserts, evaluated the sensory quality of the prepared samples. The panelists were presented with six coded dairy dessert samples (40 g) developed with different concentrations of date syrup, date powder, and sugar, respectively, according to the combinations generating by the mixture design: 2–14–0% (MDD_1), 2–2–12% (MDD_2), 2–2–12% (MDD_3), 2–14–0% (MDD_4), 14–2–0% (MDD_5), and 14–2–0% (MDD_6), and two other formulations developed with date syrup 16–0–0% (SDD) and date powder 0–16–0% (PDD), using a 5-point hedonic scale, ranging from 5 (extremely liked) to 1 (extremely disliked), for each sensory attribute: texture, hardness, granularity, sweetness, color, odor, flavor, and savor with an evaluation of the overall acceptability on a scale of 1–9.

2.5 | Physicochemical analyses

2.5.1 | Water, lipid, protein, and sugar contents

The thermal drying process was followed for the determination of the water content of CDD, SDD, and optimal formulation of MDD as described by Boulekbache-Makhlouf, Slimani, and Madani (2013). The lipids of each sample were extracted in a Soxhlet apparatus using hexane as a solvent, boiling for 30 min (Alem, Ennassir, Benlyas, Mbark, & Zegzouti, 2017). The protein content of samples was determined in quadruplicate as %N × 6.25 using a LECO CHNS-932 nitrogen microanalyser (Leco Corporation, St Joseph, USA) (da Silva et al., 2019). As for sugars, the content of formulated desserts was deduced from that of syrup and powder, determined in our previous study using HPLC-RI (Djaoud

TABLE 1 Observed responses for the three-component D-optimal mixture design

Samples	X_1-DS	X_2-DP	X_3-Sugar	Y_1-Texture	Y_2-Hardness	Y_3-Granularity	Y_4-Sweetness	Y_5-Color	Y_6-Odor	Y_7-Savor	Overall acceptability
MDD_1	0.125	0.875	0	3.6	1.9	1.7	2.2	4.2	2.8	1.8	1.8
MDD_2	0.125	0.125	0.75	4.1	4.3	4.4	3.7	2.0	3.5	3.7	6.9
MDD_3	0.125	0.125	0.75	4.0	4.0	4.1	3.5	1.5	3.1	3.4	6.7
MDD_4	0.125	0.875	0	3.3	1.5	1.5	2.0	4.0	2.5	1.5	1.5
MDD_5	0.875	0.125	0	4.3	4.5	3.9	4.5	4.7	4.1	4.4	7.1
MDD_6	0.875	0.125	0	4.1	4.3	3.7	4.3	4.5	3.9	4.1	7.2
SDD	1	0	0	4.8	4.6	4.6	4.8	4.6	4.6	4.4	7.6
PDD	0	1	0	3.1	1.7	1.5	2.3	3.6	1.7	1.6	1.6

et al., 2019). However, that of the commercial product (CDD) was fixed by the company Soummam Dairy (company-specific standard).

2.5.2 | Electrophoresis SDS-PAGE

To confirm the modification (i.e., cross-linking) of milk proteins in the formulated new products: SDD and optimal formulation of MDD in comparison with CDD, a sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed following the methodology described by Laemmli (1970). A mini Protein Electrophoresis Cell (Bio-Rad Laboratories, California, USA) and a discontinuous system composed of a stacking gel (4 g/100 g) and separating gel (10 g/100 g) were used for the identification of the cross-linking proteins. After the run, the gel was stained with the dye Coomassie Blue R-250 (1.92 g) in a mixture of methanol (40 ml), glacial acetic acid (160 ml), and distilled water (400 ml). For the decolorization of the gel, a solution containing methanol (200 ml), ethanol (100 ml), glacial acetic acid (50 ml), and distilled water (300 ml) was prepared. The protein fractions were identified using the molecular weight standard (Protein Plus Protein Standards).

2.6 | Total phenolic content (TPC) and antioxidant activity

Phenolic compounds were extracted according to the method described by Amerinasab, Labbafi, Mousavi, and Khodaiyan (2015). The results were expressed as mg gallic acid equivalent (GAE) per 1 g of the dry weight (DW): mg GAE/g DW. The ability of the extracts to scavenge DPPH-free radicals was determined by the method used by Milardović, Iveković, and Grabarić (2006). The reducing power was determined according to Oyaizu (1986) method, the results were expressed as mean values ± standard deviations.

2.7 | Microbiological tests

The count of the total flora and the search for undesirable microorganisms (coliforms, fecal coliforms and *Staphylococcus aureus*) were performed according to a standard procedure (Marshall, 1993). Total flora was determined using plate count agar with an incubation at 32°C for 48 hr. Presumptive coliforms were enumerated on purple red bile agar after incubation at 37°C for 24 hr. *Staphylococcus aureus* was determined using the Baird–Parker agar supplemented with tellurite, incubated at 35°C for 48 hr.

2.8 | Rheological measurements

Rheological measurements of CDD, SDD, and optimal formulation of MDD were performed, as described by Zarzycki, Ciołkowska, Jabłońska-Ryś, and Gustaw (2019), in a controlled stress rheometer (AR2000, TA Instruments USA) using parallel plate geometry (dia: 60 mm diameter; 1 mm gap) a rough surface in order to avoid slipping effects and a gap of 1 mm.

2.8.1 | Steady-state shear measurements

Steady-state shear flow test was performed from 1 to 200 s^{-1} for the three samples. The results were adjusted to the Ostwald-de Waele or Power-law model, which is one of the methods known for explaining the behavior of fluids. The model applies the following expression:

$$\eta = K^{n-1}\gamma \tag{1}$$

where η is the apparent viscosity, $\dot{\gamma}$ is the shear rate, and K and n are the consistency and flow index, respectively. The apparent viscosity (η) at the intermediate shear rate ($\dot{\gamma}$ = 0.01 s^{-1}) and flow index (n) were the parameters selected for comparison. The flow index (n) is selected because it determines how the flow precisely develops, if $n < 1$ the fluids called pseudo-plastic, these fluids flow more easily by increasing shear rate (shear-thinning). Moreover, when $n > 1$, the flow resistance increases with increased shear rate and it is called a dilatant fluid (shear-thickening). In order to compare, viscosity values at 10 s^{-1} (η_{10}) were selected.

2.8.2 | Viscoelastic behavior

Stress sweep tests were made between 0.2 and 300 Pa, at a frequency of 1 Hz (6.28 rad/s), in all the systems studied to determine the linear viscoelasticity region and critical stress. Frequency sweeps tests at 0.05 Pa, which is within the linear viscoelastic region since this value (0.05 Pa) is lower than the critical stresses for all the systems, were performed from 0.01 to 100 rad/s. Storage and loss moduli (G′ and G′′, respectively) are plotted as a function of frequency. The oscillatory rheological parameters used to compare the viscoelastic properties of the samples were storage modulus (G′) and loss tangent (tan δ = G′′/G′) at 2 rad/s (G'_2 and tan δ_2).

2.9 | Confocal laser scanning microscopy (CLSM)

The microstructure of CDD, SDD, and optimal formulation of MDD was observed using confocal laser scanning microscopy (CLSM) in the reflectance mode, as described by Kermiche et al. (2018), using a ZEISS LSM7 DUO (Germany) microscopy, with an Argon laser at 488 nm, and a detection between 493 and 619 nm. The microstructure images were analyzed using image analysis software (ZEN2012SP1).

2.10 | Statistical analysis

Each analysis was carried out in triplicate and all the data have been reported as means ± standard deviation (*SD*), statistically assessed by ANOVA analysis of variance and Tukey's post hoc test with a 95% confidence level, using XLSTAT Release 10 (Addinsoft, Paris, France).

3 | RESULTS AND DISCUSSION

3.1 | Analysis of D-optimal mixture experiments and validation of models

A three-factor, two-level D-optimal mixture design was used in this study to correlate the effect of formulation ingredients (X_1-Date syrup, X_2-Date powder, and X_3-Sugar) with the observed responses (Y_1-Texture, Y_2-Hardness, Y_3-Granularity, Y_4-Sweetness, Y_5-Color, Y_6-Odor, and Y_7-Savor). The lead number of experiments ($n = 6$) was suggested by the Design-Expert software, and runs were randomly set. The independent and dependent design variables are listed in Table 1, with the experimental runs and the observed responses. After producing and analyzing the formulations, sensory responses were applied to fit the appropriate model (linear).

The model was tested for goodness of fit (R^2) and analysis of variance (ANOVA) was applied to verify the adequacy of the regression models in terms of a lack-of-fit test. This test implies that the residual response sum of squares is separated into the components model error and pure error, and their significances were obtained by an *F* test (Table 2). The probability value (*p*-value) for the determination of statistical significance was set at .05, which indicates that a "hypothesis" theory would be rejected if the calculated *p* value was less than .05 in favor of an alternative theory.

As shown in Table 2, the linear model was statistically significant (*p*: .0316, .0020, .0011, .0011, .0023, .0200, and .0025, respectively), which indicates that the model adequately describes the responses. The lack-of-fit test was subsequently performed to further demonstrate the suitability of a given model. Lack-of-fit test, which was calculated based on the residual sum of squares, diagnoses whether a model adequately fits the data. As seen in Table 2, a large *p* value of the linear model ($p > .05$) for Y_1 (0.36), Y_2 (0.44), Y_3 (0.27), Y_4 (0.42), Y_5 (0.13), Y_6 (0.26), and Y_7 (0.42), respectively, indicates that the linear model adequately fits the data.

The values of R^2 (0.90, 0.98, 0.99, 0.99, 0.98, 0.93, and 0.98) and adjusted R^2 (0.83, 0.97, 0.98, 0.98, 0.97, 0.98, and 0.97) indicated that the experimental data were in a good agreement with predicted values.

Considering the data shown in Table 2, mathematical predictive models of mixture ratio of date syrup, date powder and sugar on the sensory characteristics of the dairy dessert formulations can be developed. Mathematical predictive models are as follows:

$$Y_1(\text{Texture}) = 4.2X_1 + 3.45X_2 + 4.05X_3 \quad (2)$$

$$Y_2(\text{Hardness}) = 4.4X_1 + 1.7X_2 + 4.15X_3 \quad (3)$$

$$Y_3(\text{Granularity}) = 3.8X_1 + 1.6X_2 + 4.25X_3 \quad (4)$$

$$Y_4(\text{Sweetness}) = 4.4X_1 + 2.1X_2 + 3.6X_3 \quad (5)$$

$$Y_5(\text{Color}) = 4.6X_1 + 4.1X_2 + 1.75X_3 \quad (6)$$

$$Y_6(\text{Odor}) = 4X_1 + 2.65X_2 + 3.3X_3 \quad (7)$$

$$Y_7(\text{Savor}) = 4.25X_1 + 1.65X_2 + 3.55X_3 \quad (8)$$

where X_1, X_2 and X_3 denote date syrup, date powder, and sugar, respectively.

According to equations (2)–(8), the mathematical predictive models were first-order regression model, with no interaction term, so the studied factors have a significant impact to the formulation of dairy desserts and X_1 (i.e., date syrup) was the most significant factor.

The effect of the independent variables on sensory characteristics of the dairy dessert formulations can be implemented by a triangular-dimensional contour plot. Figure 1 describes the variation on the responses as a function of the mixture composition and indicates that sensory characteristics enhance mainly by an increased ratio of date syrup (X_1). A lower quantity of date powder and sugar (X_2 and X_3) is better for the sensory quality of the MDD formulations. The optimum mixture of dairy dessert could provide more nutrition for consumers with the total substitution of sugar by date syrup and powder.

Additional experiments with optimal conditions (0.875 for date syrup, 0.125 for date powder, and 0 for sugar) were carried out in order to assess the validity of the model. The experimental values (4.0, 4.6, 3.8, 4.5, 4.9, 4.3, and 4.3) were found to be reasonably close to the predicted ones (4.2, 4.4, 3.8, 4.4, 4.6, 4.0, and 4.3), which confirmed the validity and adequacy of the predicted models. These results confirm the excellent reproducibility of the experimental conclusion.

3.2 | Sensory evaluation

Understanding how consumers perceive dairy foods is critical for the food industry. This information is essential for the development and marketing of new products, the reformulation of existing ones, and the optimization of manufacturing processes. Apart from knowing how much consumers like a product, in order to design food products that meet consumer sensory expectations, information about which sensory characteristics consumers expect to find in the product are needed, that is, which sensory attributes drive consumer liking. As consumers are not always able to say why they like a product, affective, descriptive and projective sensory methods have been widely used in several studies to answer this question (Pinto et al., 2018; Torres et al., 2017).

Consumers' affective test, such as the test run in this study, provide useful information on the future commercial potential of new

TABLE 2 Analysis of variance (ANOVA) for the regression model

Source	Sum of squares	DOF	Mean square	*F*-value	*p*-value	Sum of squares	DOF	Mean square	*F*-value	*p*-value
	Y_1-Texture					Y_2-Hardness				
Model	0.63	2	0.32	13.50	.0316*	8.90	2	4.45	92.10	.0020*
X_1-Date syrup	35.28	1		1512.00	<.0001*	38.72	1		801.10	<.0001*
X_2-Date powder	23.81	1		1020.21	<.0001	5.78	1		119.59	.0016*
X_3-Sugar	32.81	1		1405.93	<.0001*	34.45	1		712.61	.0001*
Lack-of-Fit	0.01	1	0.01	1.15	.73	0.05	1	0.05	0.90	.44
Residual	0.07	3	0.02			0.15	3	0.05		
Pure Error	0.07	1	0.03			0.10	2	0.05		
R^2	0.90					0.98				
R^2_{adj}	0.83					0.97				
Cor total	0.70	5				9.05	5			
	Y_3-Granularity					Y_4-Sweetness				
Model	8.04	2	4.02	141.94	.0011*	5.45	2	2.73	136.33	.0011*
X_1-Date syrup	28.88	1		1019.23	<.0001*	38.72	1			<.0001*
X_2-Date powder	5.12	1		180.71	.0009*	8.82	1			.0002*
X_3-Sugar	36.13	1		1275.00	<.0001*	25.92	1			<.0001*
Lack-of-Fit	0.05	1	0.05	0.25	.27	0.02	1	0.02	1.00	.42
Residual	0.09	3	0.03			0.06	3	0.02		
Pure error	0.04	2	0.02			0.04	2	0.02		
R^2	0.99					0.99				
R^2_{adj}	0.98					0.98				
Cor total	8.13	5				5.51	5			
	Y_5-Color					Y_6-Odor				
Model	9.26	2	4.63	84.21	.0023*	1.82	2	0.91	18.86	.0200*
X_1-Date syrup	42.32	1		769.45	.0001*	32.00	1		662.07	.0001*
X_2-Date powder	33.62	1		611.27	.0001*	14.05	1		290.59	.0004*
X_3-Sugar	6.13	1		111.36	.0018*	21.78	1		450.62	.0002*
Lack-of-Fit	0.13	1	0.13	6.25	.13	0.08	1	0.08	2.46	.26
Residual	0.17	3	0.06			0.15	3	0.05		
Pure Error	0.04	2	0.02			0.07	2	0.03		
R^2	0.98					0.93				
R^2_{adj}	0.97					0.88				
Cor total	9.43	5				1.97	5			
	Y_7-Savor									
Model	7.24	2	3.62	80.44	.0025*					
X_1-Date syrup	36.13	1		802.78	<.0001*					
X_2-Date powder	5.45	1		121.00	.0016*					
X_3-Sugar	25.21	1		560.11	.0002*					
Lack-of-Fit	0.05	2	0.05	1	.42					
Residual	0.14	3	0.05							
Pure error	0.09	2	0.05							
R^2	0.98									
R^2_{adj}	0.97									
Cor total	7.38	5								

developed food (Cardarelli, Buriti, Castro, & Saad, 2008). Table 1 shows the means sensory ratings for dairy desserts, ranged from extremely disliked to extremely liked (from 1 to 5, respectively), containing varying percentages of date syrup, date powder, and sugar, respectively, 2–14–0% (MDD_1), 2–2–12% (MDD_2), 2–2–12% (MDD_3), 2–14–0% (MDD_4), 14–2–0% (MDD_5), 14–2–0% (MDD_6), 16–0–0% (SDD), and 0–16–0% (PDD). There were significant differences in texture, hardness, granularity, sweetness, color, odor, savor, and overall acceptability between samples. The minimum scores were attributed to powder dairy dessert (PDD), which might be due to the high content of date powder (16%) providing intense astringency taste. However, SDD was the most appreciated by consumers. Jridi et al. (2015) have found that an increase in the quantity of syrup leads to reduce the astringent appearance in the final product.

In addition, when comparing the mixture dairy dessert formulations: MDD_2, MDD_3, MDD_5, and MDD_6, samples with 2% of date powder were the best scored compared with MDD_1 and MDD_4 with 14% of date powder. Regarding the data in Table 1, the panelists judged negatively the addition of date powder with high concentration (14%). In the case of date syrup and sugar, their use for the enrichment of dairy desserts improved the scores for all the descriptors.

Our study demonstrates that the substitution of 16% sugars in dairy dessert by date syrup and powder as suitable sweeteners ingredients is possible. According to the results of mixture design, the best formulation of MDD was: 14% date syrup, 2% date powder, and 0% sugar concentrations. Based on the obtained results, SDD and optimal formulation of MDD were selected to evaluate the possible influence of date syrup and powder (DS and DP) incorporation on their physicochemical, phytochemical, microbiological, rheological and microstructural characteristics, and the results were compared with a commercial dairy dessert CDD.

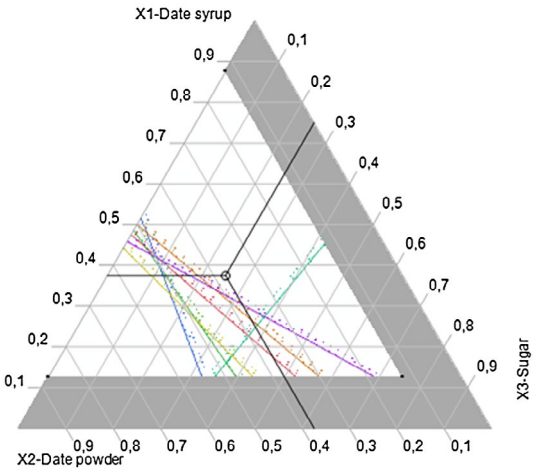


FIGURE 1 Triangular-dimensional contour plot illustrating the effect of date syrup (X_1), date powder (X_2), and sugar (X_3) on the responses

3.3 | Physicochemical analyses

3.3.1 | Water content

The presence of huge available water quantities in the components is the principal factor inducing the development of bacteria, which affects safety and food quality. From Table 3, a significant difference ($p < .05$) was observed between the water content of different dairy desserts: 77.84 ± 0.16^b%, 76.98 ± 0.06^c%, and 78.48 ± 0.12^a% for SDD, optimal formulation of MDD, and CDD, respectively. Dairy desserts with date syrup and powder were characterized by the lowest water content compared to the CDD, this result could be explained by the greater richness of dates (Degla-Beida variety) in fibers. In fact, the presence of such components increased water retention in the corresponding product. Furthermore, among the two formulated samples, the highest level shown in SDD might be due to the high proportion of syrup in these formulation. The incorporation of date syrup and powder into dairy desserts increased its dry matter. Indeed, the dry matter of the samples was: 22.16, 23.20, and 21.77% for SDD, optimal formulation of MDD, and CDD, respectively. Our data were in the range of those reported by Nongonierma et al. (2007) for yogurts at 0 and 5% fat, added with sugar syrups which were equals to 20.80 and 25.10%, respectively. They were also close to those obtained by Amellal (2008) for yogurts enriched with date powder of different Algerian common varieties which were between 20.64 and 21.39%.

3.3.2 | Lipid content

A large percentage of fat in dairy products is saturated compounds but they are also a source of fat-soluble vitamins and unsaturated fats, including omega-3s (Canada's Food Guide, 2011). So, milk fat contains fatty acids which are considered essential nutrients in the diet that can affect human health (Hanuš, Samková, Křížová, Hasoňová, &

TABLE 3 Physicochemical analyses results of CDD, SDD, and MDD

	CDD	SDD	MDD
Water content (%)	78.48 ± 0.12^a	77.84 ± 0.16^b	76.98 ± 0.06^c
Proteins (%)	2.65 ± 01^b	2.66 ± 0.00^b	3.14 ± 0.14^a
Lipids (%)	1.44 ± 0.12^b	2.15 ± 0.16^a	2.16 ± 0.13^a
Sugars (%)			
Sucrose	16.00^a	8.02^b	8.03^b
Glucose	00.00^b	3.95^a	3.87^a
Fructose	00.00^b	4.10^a	4.16^a
TPC (mg/g)	0.27 ± 0.00^c	0.65 ± 0.04^a	0.38 ± 0.03^b
DPPH (%)	2.54 ± 0.46^b	4.00 ± 0.82^a	3.22 ± 0.44^{ab}
Reducing power (mg/g)	0.17 ± 0.16^c	2.75 ± 0.10^a	2.21 ± 0.17^b

Note: Values are mean ±95% confidence interval. Values with different letters (a-b-c) were significantly different (Tukey, $p < .05$).

Kala, 2018). Lipids provide twice as much energy as the same quantity of carbohydrates and proteins (Ehirim & Onyeneke, 2013). Table 3 shows that the fat content of dairy dessert samples ranged between 1.44 and 2.16% and these percentages were almost identical for SDD (2.16 ± 0.13[a]%) and the optimal formulation of MDD (2.15 ± 0.16[a]%) compared to the CDD (1.44 ± 0.12[b]%). These values were lower than those found by Ozer, Robinson, Grandison, and Bell (1998), which varies between 4.5% and 8.2%. Moreover, they are in coincidence with those found by Amellal (2008) with values ranged from 2.12 to 3.95%. This difference may be due to a difference in the fat composition of milk and fruits. The low lipid content in relation to its sugar content means that dairy desserts enriched with dates show a better nutritional quality being healthier.

3.3.3 | Protein content

Proteins are essential for the functioning of living cells and correspond to an important part of milk and dairy products (Lapointe-Vignola, 2002). The protein content of Algerian dry dates is generally between 1.80 and 3.60% (Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005). The total protein contents of samples are shown in Table 3 with percentages ranging from 2.65 ± 01[b] to 3.14 ± 0.14[a]%. The highest content was recorded for the optimal formulation of MDD enriched with a mixture of 14% date syrup DS and 2% date powder DP (3.14 ± 0.14[a]%). These values were significantly lower ($p < .05$) for syrup and commercia dairy desserts (2.66 ± 0.00[b] and 2.65 ± 0.01[b]%, respectively). This result might be due to the incorporation of date powder into MDD which enhanced its protein content. For comparative purposes, we can say that our results agreed with those obtained by Jridi et al. (2015) who observed the enrichment of dairy desserts, in proteins, with Tunisian date syrup and powder. These data were also in agreement with those found by Ozer et al. (1998) and Fernandes, Oliveira, and Lima (2007), who reported values between 3.60–5.00 and 3.57–3.28%, respectively.

3.3.4 | Sugar content

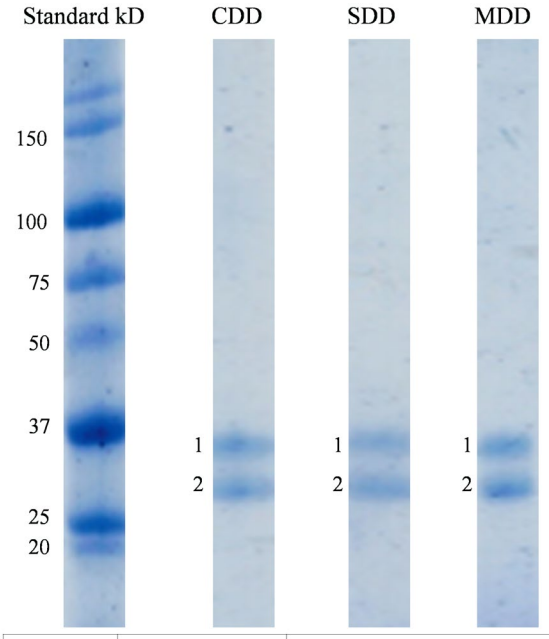
Table 3 represents the average contents of sucrose, glucose, and fructose for each sample. The obtained results showed an extensive range of variation among the three sugars. The content of sucrose (8.02[b] and 8.03[b]%), glucose (3.95[a] and 3.87[a]%), and fructose (4.10[a] and 4.16[a]%) was found in syrup and mixture dairy desserts (SDD and MDD), respectively. The predominant sugar in all of the examined samples was determined as sucrose, followed by glucose and fructose compared to that of the CDD sample, which was constituted by sucrose only (16%). To the best of our knowledge, few studies have been carried out on the individual sugar content of dairy desserts. Ahmed, Nawel, Ghalem, Zouaoui, and Djilali (2016) have been reported that the total substitution of cacao by date powders, from H'lowa variety, in dairy creamed dessert, increases the reducing sugars from 14.40% to 16.59%. These values are close to those obtained in our study.

3.3.5 | Electrophoresis analysis

Typical electropherograms of dairy desserts (CDD, SDD, and MDD) are shown in Figure 2. Two protein fractions were quantified at 29 and 32 kDa, which correspond to caseins β and α, respectively. No differences in the electrophoretic patterns were observed in the three samples, since repeatability of migration times was observed when we compared CDD, SDD, and optimal formulation of MDD. These results show that the incorporation of date syrup and powder did not promote the appearance of new high molecular-weight protein bands. The no-formation of these bands was accompanied by a high intensity of the casein bands, indicating that no proteins were formed by the cross-linking of low-molecular-weight polymers. This result is in accordance with that obtained by Oliveira et al. (2015), where caseins' fraction had no apparent differences between white yogurt and strawberry-enriched yogurt.

3.4 | Phytochemical analyses and antioxidant activity

Regular intake of fruits and vegetables is related to the reduced risk of diseases because of their richness on phenolic compounds, which are products of secondary metabolism in plants and are good sources of natural antioxidants in human diets. They play an important role in delaying the development of chronic diseases such as



Band	MD (kD)	Protein fraction
1	32	α-casein
2	29	β-casein

FIGURE 2 SDS-PAGE electrophoretogram of molecular weight markers as well as CDD, SDD, and MDD protein fractions with an enclosed table of molecular weight (MW) and protein fraction name for each signal

cardiovascular diseases, cancer, inflammatory bowel syndrome, and Alzheimer's diseases (Zhang, Yeh, Kuroki, & Wang, 2005). So, for their effects on human health, many researches have been focused in their mode of application in food industry.

The incorporation of date syrup DS and date powder DP in dairy desserts (SDD and MDD, respectively) significantly improved their phenolic contents ($p < .05$) compared with the CDD by the enhancement of their antiradical and reducing power activities. The results of total phenolic contents (TPC) and antioxidant activity of dairy dessert samples are shown in Table 3. Syrup dairy dessert showed the highest concentration of total phenolic content. In fact, the addition of 16% of date syrup in SDD, and a mixture of 14% of date syrup, and 2% of date powder in MDD, lead to an increase in TPC with an amount of 0.65 ± 0.04^{a} and 0.38 ± 0.03^{b} mg/g, respectively, in comparison with the CDD (0.27 ± 0.00^{c} mg/g). The increase of the phenolic content in the formulated dairy desserts is due to their enrichment with date by-products. Indeed, this fruit is a good source of bioactive molecules (Biglari, AlKarkhi, & Easa, 2008; Hamad et al., 2015). Indeed, the increase in the polyphenols concentration in dairy products enriched with various fruits has been reported in the literature (Amal, Eman, & Nahla, 2016; Kermiche et al., 2018).

A linear correlation was visibly observed between the total antioxidant activity and phenolic contents. In fact, the highest effect was exhibited by SDD with 4.00 ± 0.82^{a}% for antiradical activity and 2.75 ± 0.10^{a} mg/g for reducing power. However, MDD and CDD showed a DPPH inhibition of 3.22 ± 0.44^{ab} and 2.54 ± 0.46^{b}%, and a reducing power of 2.21 ± 0.17^{b} and 0.17 ± 0.19^{c} mg/g, respectively. Therefore, the addition of date syrup and powder in dairy desserts as a substitute for crystallized sugar led to a dairy desserts with high phenolic content and antioxidant activity.

Our results were in agreement with those obtained by Ivanova, Petkova, Balabanova, Ognyanov, and Vlaseva (2018), Jridi et al. (2015), and Szwajgier and Gustaw (2015), who observed the enrichment in total phenolic content and the enhancement of the antioxidant activity of dairy desserts adding with encapsulated cornelian cherry, chokeberry and blackberry juices, date syrup and powder of different Tunisian varieties, and brewery malts, respectively. Amerinasab et al. (2015), Trigueros, Sayas-Barberá, Pérez-Álvarez, and Sendra (2012) and Gad, Kholif, and Sayed (2010), noted the same thing in yogurt based on date liquid sugar, yogurts manufactured with dates blanching water, and yogurt resulting from the combination of date palm syrup and skim milk, respectively. Moreover, many studies have reported the enhancement of the antioxidant power of dairy products supplemented with fruits and vegetables (Felfoul, Borchani, Samet-Bali, Attia, & Ayadi, 2017; Kermiche et al., 2018; Sah, Vasiljevic, McKechnie, & Donkor, 2015).

3.5 | Microbiological tests

Dairy desserts are popular dairy food, prepared with ingredients including milk as a basic constituent. They are palatable and nutritious, consumed by a wide range of people of all ages and are usually served cooled. Milk-based products are good media for the growth of microorganisms because of their high nutritive value (Amin, 2018). Therefore, thermal processing can afford a high degree of food security, but it causes thermal degradation in food quality including loss of flavor and nutrition quality. In this study, the formulated dairy desserts were heat-treated or pasteurized at 90°C for 5 min. This treatment has a significant effect on the microbial flora as it destroys pathogenic and undesirable germs.

According to the results of the microbiological analysis, we distinguished a value <1 CFU/g for the total aerobic flora, with an absence of total and fecal coliforms as well as *S. aureus* in dairy desserts enriched with date syrup (SDD), the dairy dessert enriched with date syrup and powder (MDD), and in the comercial dairy dessert (CDD). These results are in concordance with the statement of Mac Graw (1797), who reported that processed milk should contain no trace of coliforms. The absence of coliforms indicates adequate heating treatment of milk and high hygienic standards of processing and packaging that precluded recontamination of the product. These results also confirm the respect of aseptic conditions during the storage of the final product.

3.6 | Rheological measurements

3.6.1 | Steady-state shear properties

Relating to the steady-state rate profiles of all dairy dessert samples, shown in Figure 3, these samples exhibited a shear thinning behavior, in other words, the shear stress increase, and consequently, the viscosity decrease with an increase in shear rate (Karaca, Güven, Yasar, Kaya, & Kahyaoglu, 2009). In fact, the data curve of the viscosity versus the stress shear for all samples were adequately fitted by the Ostwald-de Waele model ($R^2 > 0.99$) and the consistency index (K) and flow behavior index (n) of dairy dessert samples were calculated and are shown in Table 4.

Although all samples showed a shear-thinning behavior, clear differences were observed among them, depending on the presence or not of date syrup and powder. It can be observed that SDD and MDD

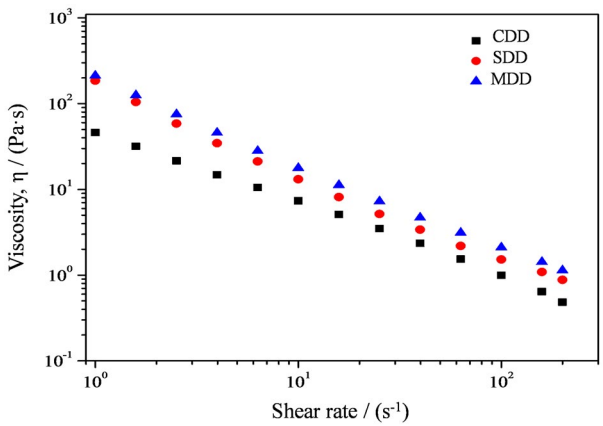


FIGURE 3 Steady shear flow curves (viscosity vs. shear rate) for CDD, SDD, and MDD

TABLE 4 Average values of apparent viscosity at 10 s^{-1}(η_{10}), consistency index (K), flow index (*n*), storage modulus (G'_2), and loss tangent (tan δ) of CDD, SDD, and MDD

	CDD	SDD	MDD
η_{10} (Pa·s)	7.34 ± 0.15[c]	13.10 ± 0.80[b]	17.70 ± 3.20[a]
K (Pa·s^n)	49.13 ± 0.94[c]	124.55 ± 11.80[b]	194.53 ± 30.13[a]
n	0.15 ± 0.01[a]	0.03 ± 0.02[b]	0.03 ± 0.02[b]
G'_2 (Pa)	151.42 ± 19.12[b]	1913.97 ± 62.93[a]	1893.63 ± 45.43[a]
tan δ_2	0.21 ± 0.00[a]	0.18 ± 0.00[b]	0.18 ± 0.00[b]

Note: Values are mean ±95% confidence interval. Values with different letters (a-b-c) were significantly different (Tukey, $p < .05$).

showed rather a similarity in the flow curves unlike CDD, whose viscosity was clearly lower. In fact, these differences can be observed in η_{10} and *K* values shown in Table 4, where significant different values between these parameters (η_{10} and *K*) were found. The more viscosity found in MDD could be attributed to the presence of date powder. In addition, syrup and MDD samples were more sensitive to the shear rate since the flow index (*n*) was almost 0 in both cases (0.03).

Therefore, the presence of date syrup and powder lead to samples with higher viscosity but more sensitive under the effect of the shear. These behaviors can be related to changes in the microstructure as a consequence of the components included by the date syrup and powder. The increase in viscosity is due to the complex composition of dates, including the presence of pectin (stabilizing agent) and mineral salts. Several authors have studied the effects of stabilizers on the thixotropic behavior of dairy products. The addition of stabilizers at high concentrations increased the thixotropy of liquid yogurt. However, the salt in milk drinks is added to reduce casein particle aggregation and apparent viscosity (Köksoy & Kılıç, 2003).

3.6.2 | Viscoelastic properties

Figure 4 shows the frequency sweeps corresponding to the CDD, SDD, and MDD. In all cases, the mechanical spectra obtained for samples correspond to a weak viscoelastic gel with a predominant elastic behavior, G′ values higher than G′′ ones, and both moduli showing slight variation with frequency (although a more dependence of G′′ values with frequency could be observed, showing the large contribution of the viscous component to higher frequencies). These results indicated that the interactions among ingredients influenced the rheological characteristics of dairy desserts. In addition, syrup and mixture dairy dessert samples (SDD and MDD) were, at first sight, the ones with the strongest gel structure, G′ and G′′ had very similar values, indicating the similarity in the structure of the two samples. Once again, samples with date syrup and powder show a more structured system. In fact, this behavior was proven by the higher values of G'_2 and lower tan δ_2 for the syrup and mixture dairy desserts (SDD and MDD) in comparison with CDD as shown in Table 4.

These variations in the dynamic parameters are due to the differences in composition (pectin content, sugar content, and mineral content "Ca^{2+} precisely") and to the difference in technological parameters (degree of homogenization, temperature and heat treatment time) between the formulated dairy desserts (SDD and MDD) and the CDD. Moreover, Norziah, Kong, Karim, and Seow (2001) investigated the effects of pectin–sucrose–Ca^{+2} interactions on rheological properties. They found that increasing the concentration of Ca^{+2}, sucrose, and pectin increases G′ and G′′, and that pectin plays a big role.

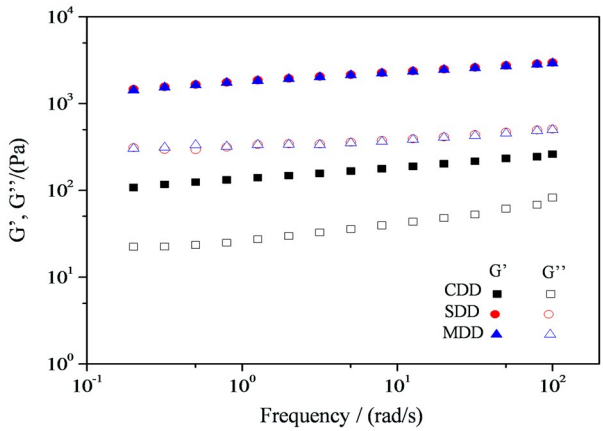


FIGURE 4 Mechanical spectra (G′ and G′′ versus frequency) for CDD, SDD, and MDD

3.7 | Confocal scanning laser microscopy (CLSM)

In food science, it is well known that the structure of foods affects significantly their properties: texture, functionality, and appearance (Puri, Khamrui, Tomar, & Lule, 2017). The microstructure has a main impact on the texture and other physical properties of milk gels (Karam, Gaiani, Hosri, Hussain, & Scher, 2016). The confocal scanning laser microscopy (CLSM) was used to observe the microstructure of milk gels, such as dairy desserts (Godoi, Bhandari, & Prakash, 2017). Figure 5 shows the microstructures of CDD, SDD, and MDD. First, for the CDD, it appears homogenous, with relatively small and uniformly dispersed pores, and a network consisting of moderately thin strands. Syrup and mixture dairy desserts (SDD and MDD) conversely, showed slightly large pores, which are linked to the presence of sugars, and the protein network appeared to be made up of rather thick strands consisting of densely aggregated protein particles. The milk used in our experiments for the preparation of syrup and mixture dairy desserts presented protein

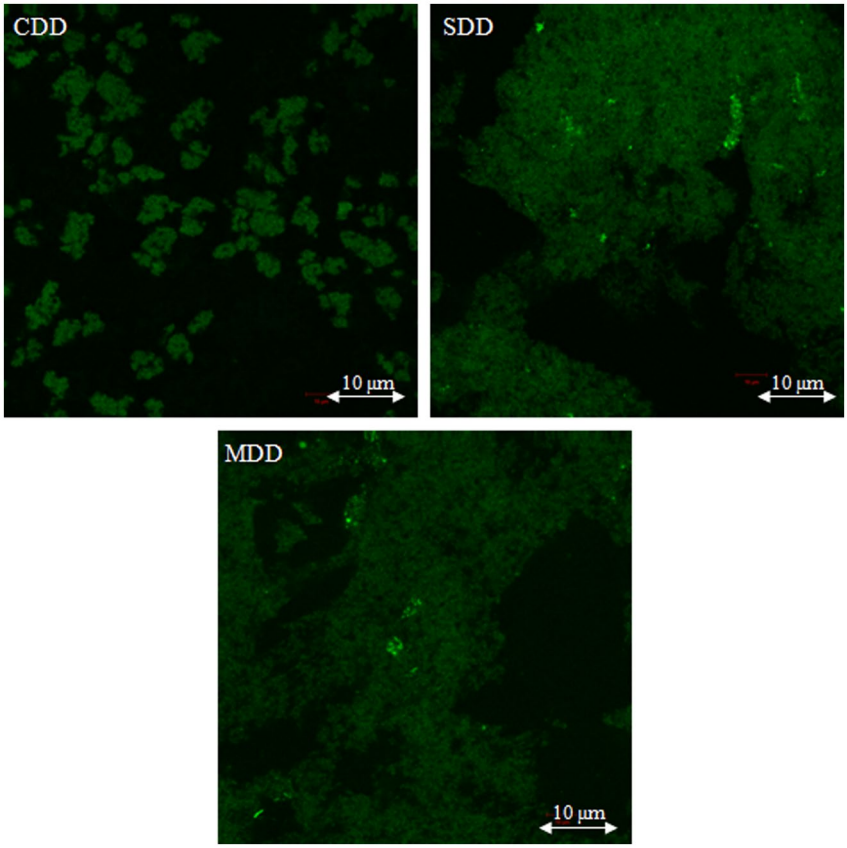


FIGURE 5 Microstructure of CDD, SDD, and MDD obtained by CLSM

particles that consist of caseins' micelles with associated whey protein. More dense and larger protein aggregates can be predicted between these particles and sugars present in date syrup and powder. This aggregation would cause gelation to occur and this fact might allow for more rearrangement to take place after the gel point, again favoring the formation of a more densely aggregated structure.

4 | CONCLUSIONS

The application of D-optimal mixture design was effective in optimizing the concentrations of date syrup and powder in dairy dessert formulation, based on its sensory evaluation. The physicochemical, phytochemical, microbiological, rheological, and microstructural properties of SDD and MDD, studied in this work, were significantly affected by both date syrup and powder additions, compared to the CDD. This incorporation enhanced the dry matter, lipids, proteins, total phenolic content, and antioxidant activity of the formulated dairy desserts, with an absence of protein cross-linking and a similarity in the structure of both syrup and mixture dairy desserts. Samples showed a high microbiological quality and a pseudo-plastic behavior. These results lead to conclude that the incorporation of date by-products in dairy dessert formulations to substitute sugar, might be an efficient alternative to formulate new products, with novel characteristics and could be qualified as functional foods with good acceptability by the consumers.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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REFERENCES

Abbès, F., Kchaou, W., Blecker, C., Ongena, M., Lognay, G., Attia, H., & Besbes, S. (2013). Effect of processing conditions on phenolic compounds and antioxidant properties of date syrup. *Industrial Crops and Products*, *44*, 634–642. https://doi.org/10.1016/j.indcrop.2012.09.008

Ahmed, H., Nawel, O., Ghalem, R. B., Zouaoui, B., & Djilali, B. (2016). Effect of total or partial substitution of cacao and sucrose by date powders variety H'lowa on the some quality of dairy creamed dessert. *Journal of Applied Environmental and Biological Sciences*, *6*(7), 100–108.

Al Alawi, R., Alhamdani, M. S. S., Hoheisel, J. D., & Baqi, Y. (2020). Antifibrotic and tumor microenvironment modulating effect of date palm fruit (*Phoenix dactylifera* L.) extracts in pancreatic cancer. *Biomedicine & Pharmacotherapy*, *121*, 109522.

Alem, C., Ennassir, J., Benlyas, M., Mbark, A. N., & Zegzouti, Y. F. (2017). Phytochemical compositions and antioxidant capacity of three date (*Phoenix dactylifera* L.) seeds varieties grown in the South East Morocco. *Journal of the Saudi Society of Agricultural Sciences*, *16*(4), 350–357.

Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., & Shahidi, F. (2005). Compositional and sensory characteristics of three native sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *Journal of Agricultural and Food Chemistry*, *53*(19), 7586–7591.

Al-Shahib, W., & Marshall, R. J. (2003). The fruit of the date palm: Its possible use as the best food for the future? *International Journal of Food Sciences and Nutrition*, *54*(4), 247–259. https://doi.org/10.1080/09637480120091982

Amal, A., Eman, A., & Nahla, S. Z. (2016). Fruit flavored yogurt: Chemical, functional and rheological properties. *International Journal of Environmental and Agriculture Research*, *2*(5), 57–66.

Amellal, H. (2008). *Aptitudes technologiques de quelques variétés communes de dattes: Formulation d'un yaourt naturellement sucré et aromatisé* [doctoral thesis]. Boumerdes: M'hamed Bougara University.

Amerinasab, A., Labbafi, M., Mousavi, M., & Khodaiyan, F. (2015). Development of a novel yoghurt based on date liquid sugar: Physicochemical and sensory characterization. *Journal of Food Science and Technology*, *52*(10), 6583–6590. https://doi.org/10.1007/s13197-015-1716-4

Amin, W. F. (2018). Occurrence of *Bacillus cereus* in some milk-based desserts. *Assiut Veterinary Medical Journal*, *64*, 41–46.

Besbes, S., Drira, L., Blecker, C., Deroanne, C., & Attia, H. (2009). Adding value to hard date (*Phoenix dactylifera* L.): Compositional, functional and sensory characteristics of date jam. *Food Chemistry*, *112*(2), 406–411.

Biglari, F., AlKarkhi, A. F., & Easa, A. M. (2008). Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. *Food Chemistry*, *107*(4), 1636–1641. https://doi.org/10.1016/j.foodchem.2007.10.033

Boulekbache-Makhlouf, L., Slimani, S., & Madani, K. (2013). Total phenolic content, antioxidant and antibacterial activities of fruits of *Eucalyptus globulus* cultivated in Algeria. *Industrial Crops and Products*, *41*, 85–89. https://doi.org/10.1016/j.indcrop.2012.04.019

Canada's Food Guide. (2011). *Health Canada*. Ottawa, ON: Minister of Health Canada.

Cardarelli, H. R., Buriti, F. C., Castro, I. A., & Saad, S. M. (2008). Inulin and oligofructose improve sensory quality and increase the probiotic viable count in potentially synbiotic petit-suisse cheese. *LWT-Food Science and Technology*, *41*(6), 1037–1046. https://doi.org/10.1016/j.lwt.2007.07.001

da Silva, S. C., Fernandes, I. P., Barros, L., Fernandes, Â., José Alves, M., Calhelha, R. C., … Filomena Barreiro, M. (2019). Spray-dried Spirulina platensis as an effective ingredient to improve yogurt formulations: Testing different encapsulating solutions. *Journal of Functional Foods*, *60*, 103427. https://doi.org/10.1016/j.jff.2019.103427

Djaoud, K., Arkoub-Djermoune, L., Remini, H., Sait, S., Tazarourte, M., Hadjal, S., … Boulekbache-Makhlouf, L. (2019). Syrup from common date variety (*Phoenix dactylifera* L.): Optimization of sugars extraction and their quantification by high performance liquid chromatography. *Current Nutrition & Food Science*, *15*, 1–13.

Ehirim, F., & Onyeneke, E. (2013). Physico-chemical and organoleptic properties of yoghurt manufactured with cow milk and goat milk. *Academic Research International*, *4*(4), 245.

FAOSTAT. (2018). *Food and agriculture organization of the United Nations*. Retrieved March 8, 2019, from http://faostat.fao.org/site/339/default.aspx

Felfoul, I., Borchani, M., Samet-Bali, O., Attia, H., & Ayadi, M. (2017). Effect of ginger (*Zingiber officinalis*) addition on fermented bovine milk: Rheological properties, sensory attributes and antioxidant potential. *Journal of New Sciences*, *44*, 2400–2409.

Fernandes, A. M., Oliveira, C. A., & Lima, C. G. (2007). Effects of somatic cell counts in milk on physical and chemical characteristics of yoghurt. *International Dairy Journal*, *17*(2), 111–115. https://doi.org/10.1016/j.idairyj.2006.02.005

Gad, A., Kholif, A., & Sayed, A. (2010). Evaluation of the nutritional value of functional yogurt resulting from combination of date palm syrup and skim milk. *American Journal of Food Technology*, *5*(4), 250–259. https://doi.org/10.3923/ajft.2010.250.259

Godoi, F. C., Bhandari, B. R., & Prakash, S. (2017). Tribo-rheology and sensory analysis of a dairy semi-solid. *Food Hydrocolloids*, *70*, 240–250. https://doi.org/10.1016/j.foodhyd.2017.04.011

Gurditta, H., Patel, A. A., & Arora, S. (2019). Selection of dietary fibres for sucrose replacement in functional Chhana-murki (Indian Cottage cheese-based dessert) and their effect on sensory, physical and instrumental texture parameters. *International Journal of Dairy Technology*, *72*(4), 633–642.

Hamad, I., AbdElgawad, H., Al Jaouni, S., Zinta, G., Asard, H., Hassan, S., … Selim, S. (2015). Metabolic analysis of various date palm fruit (*Phoenix dactylifera* L.) cultivars from Saudi Arabia to assess their nutritional quality. *Molecules*, *20*(8), 13620–13641.

Hamza-Chaffai, A. (1990). Caractérisation rhéologique d'un lait gélifié chocolaté: Optimisation de la technologie de fabrication. *Le Lait*, *70*(2), 155–167. https://doi.org/10.1051/lait:1990214

Hanuš, O., Samková, E., Křížová, L., Hasoňová, L., & Kala, R. (2018). Role of fatty acids in milk fat and the influence of selected factors on their variability—A review. *Molecules*, *23*(7), 1636. https://doi.org/10.3390/molecules23071636

Hashim, I. B., Omer, T. A., & Fathelrahman, E. (2016). Consumer acceptance and marketing of date syrup ice cream in United Arab Emirates University. *Global Journal of Biology, Agriculture and Health Siences*, *5*(1), 7–11.

Ivanova, M., Petkova, N., Balabanova, T., Ognyanov, M., & Vlaseva, R. (2018). Food design of dairy desserts with encapsulated cornelian cherry, chokeberry and blackberry juices. *The Annals of the University of Dunarea de Jos of Galati. Fascicle VI. Food Technology*, *42*(1), 137–146.

Jahromi, M., & Niakousari, M. (2018). Development and characterisation of a sugar-free milk-based dessert formulation with fig (*Ficus carica* L.) and carboxymethylcellulose. *International Journal of Dairy Technology*, *71*(3), 801–809.

Jridi, M., Souissi, N., Salem, M. B., Ayadi, M., Nasri, M., & Azabou, S. (2015). Tunisian date (*Phoenix dactylifera* L.) by-products: Characterization and potential effects on sensory, textural and antioxidant properties of dairy desserts. *Food Chemistry*, *188*, 8–15. https://doi.org/10.1016/j.foodchem.2015.04.107

Karaca, O. B., Güven, M., Yasar, K., Kaya, S., & Kahyaoglu, T. (2009). The functional, rheological and sensory characteristics of ice creams with various fat replacers. *International Journal of Dairy Technology*, *62*(1), 93–99. https://doi.org/10.1111/j.1471-0307.2008.00456.x

Karam, M. C., Gaiani, C., Hosri, C., Hussain, R., & Scher, J. (2016). Textural properties of acid milk gels: Effects of dairy protein powder rehydration state. *Journal of Food Processing and Preservation*, *40*(3), 414–421. https://doi.org/10.1111/jfpp.12618

Kermiche, F., Boulekbache-Makhlouf, L., Félix, M., Harkat-Madouri, L., Remini, H., Madani, K., & Romero, A. (2018). Effects of the incorporation of cantaloupe pulp in yogurt: Physicochemical, phytochemical and rheological properties. *Food Science and Technology International*, *24*(7), 585–597. https://doi.org/10.1177/1082013218776701

Keshtkaran, M., Mohammadifar, M. A., Asadi, G. H., Nejad, R. A., & Balaghi, S. (2013). Effect of gum tragacanth on rheological and physical properties of a flavored milk drink made with date syrup. *Journal of Dairy Science*, *96*(8), 4794–4803. https://doi.org/10.3168/jds.2012-5942

Köksoy, A., & Kılıç, M. (2003). Effects of water and salt level on rheological properties of ayran, a Turkish yoghurt drink. *International Dairy Journal*, *13*(10), 835–839. https://doi.org/10.1016/S0958-6946(03)00103-1

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature*, *227*(5259), 680.

Lapointe-Vignola, C. (2002). *Science et technologie du lait: transformation du lait*. Montréal: Presses inter Polytechnique.

Mac Graw, H. (1797). *Dairy substitutes*, *Encyclopedia of sci. and tech*, 5th ed.. New York: Mac Graw Hill publishers.

Marshall, E. (1993). Epidemiology of tumors affecting the visual system. *Optometry clinics: the official publication of the Prentice Society*, *3*(3), 1–16.

Milani, E., Koocheki, A., & Golimovahhed, Q. A. (2011). Extraction of inulin from Burdock root (*Arctium lappa*) using high intensity ultrasound. *International Journal of Food Science & Technology*, *46*(8), 1699–1704. https://doi.org/10.1111/j.1365-2621.2011.02673.x

Milardović, S., Iveković, D., & Grabarić, B. S. (2006). A novel amperometric method for antioxidant activity determination using DPPH free radical. *Bioelectrochemistry*, *68*(2), 175–180. https://doi.org/10.1016/j.bioelechem.2005.06.005

Nadeem, M., Rehman, S. U., Mahmood Qureshi, T., Nadeem Riaz, M., Mehmood, A., & Wang, C. (2018). Development, characterization, and flavor profile of nutrient dense date bars. *Journal of Food Processing and Preservation*, *42*(10), e13622. https://doi.org/10.1111/jfpp.13622

Nongonierma, A. B., Cayot, P., Springett, M., Le Quéré, J.-L., Cachon, R., & Voilley, A. (2007). Transfers of small analytes in a multiphasic stirred fruit yoghurt model. *Food Hydrocolloids*, *21*(2), 287–296. https://doi.org/10.1016/j.foodhyd.2006.04.007

Norziah, M., Kong, S., Karim, A. A., & Seow, C. (2001). Pectin–sucrose–Ca_2^+ interactions: Effects on rheological properties. *Food Hydrocolloids*, *15*(4–6), 491–498. https://doi.org/10.1016/S0268-005X(01)00089-3

Oliveira, A., Alexandre, E. M., Coelho, M., Lopes, C., Almeida, D. P., & Pintado, M. (2015). Incorporation of strawberries preparation in yoghurt: Impact on phytochemicals and milk proteins. *Food Chemistry*, *171*, 370–378. https://doi.org/10.1016/j.foodchem.2014.08.107

Oyaizu, M. (1986). Studies on products of browning reaction. *The Japanese Journal of Nutrition and Dietetics*, *44*(6), 307–315.

Ozer, B. H., Robinson, R. K., Grandison, A. S., & Bell, A. E. (1998). Gelation properties of milk concentrated by different techniques. *International Dairy Journal*, *8*(9), 793–799. https://doi.org/10.1016/S0958-6946(98)00115-0

Pinto, L. D. P. F., Silva, H. L. A., Kuriya, S. P., Maçaira, P. M., Cyrino Oliveira, F. L., Cruz, A. G., … Freitas, M. Q. (2018). Understanding perceptions and beliefs about different types of fermented milks through the application of projective techniques: A case study using Haire's shopping list and free word association. *Journal of Sensory Studies*, *33*(3), e12326. https://doi.org/10.1111/joss.12326

Prasad, W., Khamrui, K., Mandal, S., & Badola, R. (2018). Effect of combination of essential oils on physicochemical and sensorial attributes of burfi in comparison with individual essential oil and BHA. *International Journal of Dairy Technology*, *71*(3), 810–819. https://doi.org/10.1111/1471-0307.12512

Puri, R., Khamrui, K., Tomar, S., & Lule, V. (2017). Effect of ingredients and processing parameters on the texture and microstructure of acid-heat coagulated dairy dessert. *Journal of Food Processing and Preservation*, *41*(2), e12841. https://doi.org/10.1111/jfpp.12841

Sah, B., Vasiljevic, T., McKechnie, S., & Donkor, O. (2015). Effect of refrigerated storage on probiotic viability and the production and stability of antimutagenic and antioxidant peptides in yogurt supplemented with pineapple peel. *Journal of Dairy Science*, *98*(9), 5905–5916. https://doi.org/10.3168/jds.2015-9450

Sayas-Barberá, E., Martín-Sánchez, A. M., Cherif, S., Ben-Abda, J., & Pérez-Álvarez, J. Á. (2020). Effect of date (*Phoenix dactylifera* L.) pits on the shelf life of beef burgers. *Foods*, *9*(1), 102.

Silva, V. L., Silva, A. C., Costa-Lima, B. R., Carneiro, C. S., & Conte-Junior, C. A. (2017). Stability of polyphenols from blueberry (Vaccinium corymbosum L.) in fermented dairy beverage. *Journal of Food Processing and Preservation*, *41*(6), e13305.

Szwajgier, D., & Gustaw, W. (2015). The addition of malt to milk-based desserts: Influence on rheological properties and phenolic acid content. *LWT-Food Science and Technology*, *62*(1), 400–407. https://doi.org/10.1016/j.lwt.2015.01.028

Torres, F. R., Esmerino, E. A., Carr, B. T., Ferrão, L. L., Granato, D., Pimentel, T. C., … Cruz, A. G. (2017). Rapid consumer-based sensory characterization of requeijão cremoso, a spreadable processed cheese: Performance of new statistical approaches to evaluate check-all-that-apply data. *Journal of Dairy Science*, *100*(8), 6100–6110. https://doi.org/10.3168/jds.2016-12516

Trigueros, L., Sayas-Barberá, E., Pérez-Álvarez, J., & Sendra, E. (2012). Use of date (*Phoenix dactylifera* L.) blanching water for reconstituting milk powder: Yogurt manufacture. *Food and bioproducts Processing*, *90*(3), 506–514.

Vayalil, P. K. (2012). Date fruits (*Phoenix dactylifera* L): An emerging medicinal food. *Critical Reviews in Food Science and Nutrition*, *52*(3), 249–271.

Wang, Q., Liu, C., Jing, Y.-P., Fan, S.-H., & Cai, J. (2019). Evaluation of fermentation conditions to improve the sensory quality of broomcorn millet sour porridge. *LWT*, *104*, 165–172. https://doi.org/10.1016/j.lwt.2019.01.037

Zarzycki, P., Ciołkowska, A. E., Jabłońska-Ryś, E., & Gustaw, W. (2019). Rheological properties of milk-based desserts with the addition of oat gum and κ-carrageenan. *Journal of Food Science and Technology*, *56*(11), 5107–5115. https://doi.org/10.1007/s13197-019-03983-4

Zhang, C.-Y., Yeh, H.-C., Kuroki, M. T., & Wang, T.-H. (2005). Single-quantum-dot-based DNA nanosensor. *Nature Materials*, *4*(11), 826. https://doi.org/10.1038/nmat1508

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ORIGINAL RESEARCH PAPER

RP-HPLC-PDA-ESI-MS/MS SCREENING OF BIOACTIVE COMPOUNDS FROM DEGLA-BEIDA DATES: CONVENTIONAL AND GREEN EXTRACTION TECHNOLOGIES

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Abstract

Date fruits have vital therapeutic properties, in the way that they represent an inexhaustible source of bioactive compounds such as polyphenols. Therefore, the objective of this work was to evaluate the phytocomposition by High Performance Liquid Chromatography (RP-HPLC-PAD-ESI-MS/MS) of the optimized microwave-assisted extraction (MAE) phenolic extract by using the response surface methodology (RSM) compared to the ultrasound-assisted and conventional extractions (UAE and CE), and to test its antioxidant activity *in vitro*. The resulting regression model indicated that a quadratic polynomial model was best suited for the spectrophotometrically determined total phenolics. The maximum total phenolic content, TPC, (4.27±0.09 mg GAE/g DW) was obtained with a 50% (v/v) ethanol's concentration, a 700 W microwave power and an extraction time of 2.42 minutes. HPLC analysis revealed the presence of organic sugars and acids, nucleoside, carotenoids, proanthocyanidins, lignans, flavonols, flavones, saponin and sterol glucosides. The MAE phenolic extract showed 58.72±0.29% DPPH radical scavenging activity and a 1.88±0.09 mg AscAE/mL reducing power.

Keywords: Dates, microwave-assisted extraction (MAE), phenolic compounds, optimization, antioxidant activity, HPLC-PAD-ESI-MS/MS analysis

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Introduction

Date palm is a fundamental species of the oasis ecosystem, its fruit (*Phoenix dactylifera* L.) being an important crop in the arid and semiarid regions of the world and is marketed globally as a high-value fruit (Abbès *et al*., 2013, Besbes *et al*., 2009, Chandrasekaran and Bahkali, 2013). The cultivars characterized by a low commercial quality known as common cultivars are less appreciated and account for approximately 30% of the Algerian dates production (Chibane *et al*., 2007). It was reported that second-grade dates (with a hard texture) from three potential Tunisian cultivars (Deglet Nour, Allig and Kentichi) contain the same levels of sugar, fiber and phenolics (Besbes *et al*., 2009).

There are thousands of naturally occurring antioxidants that belong to several different classes of compounds (*e.g.* carotenoids, polyphenols, polyamines, tannins and catechins). Polyphenols are the most abundant natural antioxidants present in plants and plant by-products. Fresh fruits and vegetables are the greatest sources of natural antioxidants and their consumption has been positively related to a decrease of the risk of chronic conditions such as cancer, atherosclerotic heart disease, neurological disorders, including Alzheimer's and Parkinson's diseases (Chu *et al.*, 2002). The phenolics with a high antioxidant activity have been identified in several agro-industrial by-products.

Microwave assisted-extraction is a recently developed technique which has been widely applied for the extraction of organic compounds from environmental samples (Camel, 2000). In the last two decades, new investigations have been prompted by an increasing demand of more efficient extraction techniques, amenable to automation. Shorter extraction times, reduced organic solvent consumption, energy and costs saved were the main tasks pursued. This technique's inherent advantages compared to the traditional conventional solid-liquid extraction techniques have drawn interest (Ballard *et al.*, 2010; Dai *et al.*, 1999; Venkatesh and Raghavan, 2004). Driven by these goals, the advances in the microwave extraction have resulted in a number of innovative techniques such as microwave-assisted solvent extraction, vacuum microwave air microwave distillation, microwave-headspace extraction, microwave hydro-diffusion and gravity, and solvent-free microwave extraction. One of the most successful stories of the twenty-first Century has been the partial replacement of conventional extraction processes with "green" procedures (reducing energy, time, solvent, and waste) based on microwave irradiation (Chemat *et al*., 2012).

The overall objective of this research was to optimize the extraction of bioactive compounds from *Phoenix dactylifera* L. by determining the optimal conditions required for the microwave-assisted extraction of total phenolic compounds (TPC) from common dates, and the comparison of the obtained results to those of the ultrasound-assisted and conventional methods, using the response surface methodology through Box-Behnken design (BBD) with a quadratic regression model. Then, the evaluation of the phytochemical and antioxidant activity of the best extracts in terms of the TPC yield of the dates' extract was also assessed through a RP-HPLC-PAD-ESI-MS/MS analysis.

Materials and methods

Chemicals and plant material

The HPLC-grade water was obtained from a LC-Pak™ Millex system (Millipore Corporation, Billerica, MA, USA). Formic acid, MS grade methanol, citric acid, galacturonic acid, malic acid, glucose, sucrose, gallic acid, and caffeine were obtained from Sigma-Aldrich, St. Louis, MO, USA. Quinic acid, catechin, procyanidin B_1, procyanidin C_1, kaempferol glucoside, kaempferol, rutin, and quercetin rhamnoside were obtained from PhytoLab, Vestenbergsgreuth, Germany.

The ‘‘Degla-Beida’’ dates used in this study present a dry nature and a hard texture, that are found in the palm groves of South-East Algeria. The choice of this variety is justified by its relative abundance on the national territory, its low market value, its long shelf life due to its dry nature, its taste and nutritional quality (energy source represented by its high sugar content) (Djaoud *et al*., 2020). The dates were washed with tap water followed by distillated water, pitted and deposited in a drying oven at 40°C until up to a constant weight. Then, the dried samples were ground into a fine powder, which was passed through a standard sieve (125 µm diameter) and only the fraction with the particle size≤125 µm was used. Finally, the powder was stored in the darkness (Djaoud *et al*., 2019).

Phytochemical analysis

Extraction of date polyphenols

Three extraction methods were used for the extraction of the dates’ polyphenols: microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and conventional extraction (CE).

Microwave-assisted extraction (MAE)

The MAE experiments for the extraction of polyphenols from *Phoenix dactylifera* L. were executed using a laboratory microwave system (MAXMOS23S, Maxipower, China). This apparatus operates at 2450 MHz with a maximum delivered power of 900 W. These experiments were conducted with date powder (1g) and solvent added (water, ethanol, methanol and acetone) at different concentrations (20, 40, 60, 80 and 100% v/v), at room temperature. The samples were treated under different selected conditions (100, 300, 500, 700 and 900 W of microwave power, and 1:20, 1:40, 1:60, 1:80 and 1:100 g/mL solvent-to-solid ratios). Indeed, the effect of the type of solvent, the ethanol/water mixture (%), the microwave power (W), the microwave irradiation time (min) and the solvent-to-solid ratio on the extraction yield of TPC were studied. After each experiment, the extract was filtered through filter paper. The parameters that had the greatest influence on the extraction of polyphenols using MAE were selected for the response surface methodology study.

Ultrasound-assisted extraction (UAE)

The ultrasound-assisted extraction of dates’ polyphenols was performed in an ultrasonic bath (mod.2510E-DTH, Mexico, USA), with the working frequency

fixed at 42 kHz, as described by Abu-Reidah *et al.* (2017) with slight modifications. 1 g of date powder was extracted with 20 mL of ethanol 50% (v/v), for 60 min at room temperature. Afterwards, the extract was paper filtered, and the volume of the solution was adjusted with ethanol and stored at 4 °C.

Conventional extraction (CE)

The dates' phenolic compounds were extracted according to the method of Al-Turki *et al.* (2010), with slight modifications. One gram of date powder was extracted with 20 mL of ethanol 50% (v/v) to minimize the solubility of sugars. The mixed ethanol-sample material system was kept under magnetic stirring on a shaker for 60 min at room temperature. The extracts were then paper filtered. The final volume of the solution was adjusted with the ethanol 50% and stored at 4°C.

Total phenolic content (TPC)

The amount of total phenolics was determined using the Folin-Ciocalteu reagent and gallic acid as a standard as described by Georgé *et al.* (2005). The results were expressed as mg gallic acid Equivalent per gram dry weight (mg GAE/g DW).

Evaluation of the antioxidant activity

Two different methods were tested: the scavenging of the DPPH radical and the reducing power. The ability of the extract to scavenge the DPPH free radical was determined by the method of Milardović *et al.* (2006), and the reducing power was assessed according to the method of Oyaizu (1986).

RP-HPLC-PAD-ESI-MS/MS analysis

The chromatographic analysis of the optimized phenolic extract, obtained by microwave-assisted extraction under the optimal conditions (X_1-Ethanol concentration, X_2-microwave power and X_3-irradiation time) suggested by the Design-Expert® software, were performed using a Thermo Finnigan Surveyor Plus HPLC apparatus equipped with a quaternary pump, a Surveyor UV-Vis PDA detector, and a LCQ Advantage max ion trap mass spectrometer (all from Thermo Fisher Scientific, Waltham, MA), coupled through an ESI source. The RP-HPLC-PAD-ESI-MS/MS data were acquired under positive and negative ionization modes, using the Xcalibur software. With this aim, the ion trap operated in full scan (100-2000 *m/z*), data dependent scan and MS^n modes; in fact, when greater discrimination was required, additional targeted MS^2 and MS^n experiments were performed on the selected pseudomolecular ions.

To optimize the MS operating conditions a preliminary experiment was performed: 10 µg/mL caffeine (H_2O/MeOH:50/50 with 0.1% formic acid) and 10 µg/mL gallic acid (H_2O/MeOH:50/50 with 0.1% formic acid) solutions were directly infused in the ESI interface at a flow rate of 25 µL/min into the mass spectrometer. The optimized conditions were as follows: 60 arbitrary units of sheat gas, a capillary temperature of 220 °C, auxiliary gas 20 and 25 spray voltage 4.5 and 5 kV, capillary voltage -26.13 V and 35 V, respectively for negative and positive ionization mode. The separation was achieved on a Kinetex XB RP18 80A (150 x 4.6 mm; 5 µm) column, protected by the corresponding guard column, both from Phenomenex,

California, USA, operating at 25 °C. A gradient elution was executed with acidified water (0.1% formic acid) as the A mobile phase and methanol as the B mobile phase, at a flow rate of 0.3 mL/min. The elution gradient for the separation of the dates extract polyphenols consisted of moving from: 10 to 70% B in 104 min, 70 to 80% B in 5 min, 80 to 100% B in 5 min, a 5 min isocratic of 100% B, 100 to 10% B in 5 min and finally an isocratic of 10% B for 6 min. The sample tray temperature was set at 4°C and the injection volume was 5 µL. The chromatograms were recorded at λ 254, 280, 330 nm and the spectral data were collected in the range of 200-800 nm for all the peaks. The compounds were characterized on the basis of their UV-Vis and mass spectra, checking the molecular ion and the fragment ions against the fragmentation patterns of the standard molecules where possible and the molecules described in the literature.

Experimental design

The response surface methodology was employed to explore the variables that affect the microwave extraction and this approach enables the overall number of experiments and possible interactions between the variables to be considered.

A Box-Behnken design was developed and a fractional three-level/three-factor experimental design with three replicates at the center point was used to investigate the effects of three independent variables (X_1-Ethanol's concentration, X_2-Microwave power and X_3-Iradiation time) on the extraction yield of the phenolic compounds from the dates' sample.

The independent variables were coded at three levels (-1, 0 and 1) and each level was selected based on the stability of the TPC under the MAE conditions. The complete experimental design consisted of 15 experimental points. The technique described above was used to obtain the surface response by fitting the data to a polynomial model and the effects of each factor while the interactions between the studied factors being also evaluated.

Validation of the optimized solution and predicted model

To verify the suitability of the quadratic equations in predicting the optimal response values, the verification experiments were carried out under optimal conditions and compared to the predicted values. The extractions under the optimal values were made in triplicate.

Statistical analysis

All data were presented as mean standard deviations (± SD) of three repeated experiments. The influence of each factor on the TPC yield in the single factor experiment for the MAE was statistically assessed by the analysis of variance (ANOVA) and the Tukey's post hoc test with a 95% confidence level using the XLSTAT Release 10 (Addinsoft, Paris, France).

Results and discussion

Phytochemical analysis

Total phenolic compounds (TPC)

Effects of extraction variables

The MAE was strongly affected by several parameters, such as the composition and the volume of the extractant, the microwave power and the extraction time.

Effect of extraction solvent

Ethanol and water are the most appropriate solvents used in the food and nutraceutical industry, because they are easily removed from the final product and they increase the efficiency of the phenolic acids and flavonoids' extraction of (Moraes *et al*., 2013). Ethanol, methanol, acetone (at 60% v/v) and distillated water were used to investigate the effect of the extraction solvent on the TPC yield as shown in Table 1. The highest TPC content was detected in the ethanol extract (2.75±0.04 mg GAE/g) followed by methanol and acetone extracts then by the distillated water extract. The obtained results led us to conclude that ethanol was significantly ($p<0.05$) the best extraction solvent in term of the TPC yield, so that consequently it was selected for the following experiments and for the RSM trial.

Effect of ethanol's concentration

The different aqueous EtOH mixtures have different dielectric properties in the MAE, and this has a profound effect on the extraction of phenolic compounds (Setyaningsih *et al*., 2015). The effect of the ethanol's concentration on the extraction efficiency was determined and the extract yields were compared (Table 1). The highest extraction yield was obtained by using 60% ethanol, this concentration was selected for the following experiments and the 40-80% range was selected for the RSM study.

Effect of microwave power

The results indicated a linear rise of the TPC yield from 100 to 700 W. This increase is because of a microwave power increase in that will improve the solubility of the sample for a better extraction efficiency. The microwave power's increment increased the dipole reactions which caused the heat generation in the mixture as a result of the power degeneration. After 700 watts, there was no increase in the yield of TPC. Consequently, 700 W was selected for the following experiments and the range 500-900 W was selected for the RSM trial.

Table 1. Preliminary study results of the microwave-assisted extraction.

Solvent		Solvent concentration		Microwave power		Ratio		Irradiation time	
Type	TPC yield ($mgGAE/g_{dw}$)	% v/v	TPC yield ($mgGAE/g_{dw}$)	W	TPC yield ($mgGAE/g_{dw}$)	ml/g	TPC yield ($mgGAE/g_{dw}$)	min	TPC yield ($mgGAE/g_{dw}$)
EtOH	2.75 ± 0.04^{a}	20	1.20 ± 0.05^{c}	100	1.92 ± 0.14^{d}	20	3.55 ± 0.10^{a}	1/2	1.17 ± 0.03^{d}
MeOH	2.12 ± 0.07^{b}	40	1.93 ± 0.07^{b}	300	2.36 ± 0.07^{c}	40	3.48 ± 0.08^{a}	1	3.03 ± 0.04^{b}
Acetone	2.13 ± 0.07^{b}	60	2.75 ± 0.04^{a}	500	2.75 ± 0.04^{b}	60	3.25 ± 0.08^{a}	2	3.26 ± 0.01^{a}
Water	1.37 ± 0.03^{c}	80	1.87 ± 0.05^{b}	700	3.38 ± 0.05^{a}	80	2.82 ± 0.14^{b}	3	2.68 ± 0.03^{c}
		100	1.10 ± 0.06^{c}	900	3.52 ± 0.06^{a}	100	2.66 ± 0.11^{b}	4	1.10 ± 0.04^{d}

Values are mean ± 95% confidence interval.
Values with different letters (a-b-c-d) were significantly different (Tukey, $p<0.05$).

Effect of irradiation time

The irradiation time is one of the key parameters which influenced the yield of TPC significantly. To study its effect, the experiments were performed for different irradiation times (1/2-5 min). The results revealed that the TPC yield increased with the irradiation time increment up to 2 minutes. Beyond 2 minutes, a decrease in the yield of TPC from *Phoenix dactylifera* L. fruit was observed. The changes may be due to the spilling of the material during the pumping associated with the longer irradiation time and the possibility of polyphenol's denaturation. The time of two minutes was selected for the following experiments and the 1-3 min range was selected for the RSM study.

Effect of solvent-to-solid ratio

The solvent to sample ratio is another important parameter which when applied correctly could decrease the mass transfer barrier and subsequently enhance the extraction yield. It can be optimized using the single factor experiment. This is because the change in the solvent's volume due to different ratio would affect the absorption of the microwave energy in MAE, making the basis of comparison inaccurate (Chan *et al*., 2014). The increase of the solvent to sample ratio at constant solvent concentration from 20 to 100 mL/g decreased the extraction yield of TPC. To avoid the solvent's wastage, the optimum solvent-to-sample ratio at a constant volume for MAE were selected and set to be 20 mL/g.

Optimization by RSM

To study the optimal conditions, an experimental design was developed for most of the dependent variables, which were the concentration of ethanol used as the extract, the microwave power and irradiation time used to develop the extraction. A 15 factorial experimental design was used to study the significance of each variable and the correlation/interaction between them (Table 2).

The combined effect of ethanol's concentration and microwave power on the TPC extraction from date fruit was shown in the surface plot in Figure 1a. The TPC extraction rate proved to be very sensitive in regards to the solvent's concentration modification. It was observed that the extraction of the TPC increased by increasing the ethanol's concentration from 40 to 60% as well as by increasing the microwave power from 300 to 700 W.

Table 2. Box-Behnken design with the observed responses of the total phenolic content (TPC) yield using the microwave-assisted extraction.

Run	X_1	X_2	X_3	Recovery of TPC (mg GAE/g)	
				Experimental	Predicted
1	60	500	120	3.69±0.01	3.62
2	60	500	120	3.56±0.03	3.62
3	60	700	180	4.00±0.06	3.98
4	80	300	120	2.75±0.03	2.73
5	80	500	180	3.12±0.02	3.19
6	80	700	120	3.24±0.04	3.20
7	40	700	120	3.93±0.08	3.95
8	40	300	120	3.40±0.03	3.44
9	40	500	60	3.45±0.05	3.39
10	60	300	60	2.93±0.05	2.96
11	60	500	120	3.61±0.02	3.63
12	40	500	180	3.77±0.08	3.78
13	60	300	180	3.73±0.06	3.68
14	60	700	60	3.59±0.01	3.64
15	80	500	60	2.51±0.05	2.51

Values are mean ± 95% confidence interval.

Nonetheless, a decreasing tendency was observed by further increasing the ethanol's concentration from 60 to 80%. From the contour plot the maximal TPC yield was predicted at the ethanol's concentration of 60% and microwave power of 700 W.

Figure 1b depict the surface plot of the effects of two variables, namely ethanol's concentration and irradiation time on the TPC yield. The ethanol's concentration demonstrated a parabolic extraction on the response which increased from 40 to 60 % and decreased from 60 to 80%. It was observed also that the extraction of TPC increased with the increase of the irradiation time as well as the ethanol's

concentration. From this contour plot, the highest values of the TPC extraction can be obtained at an ethanol's concentration of 60% and an irradiation time of 2 min.

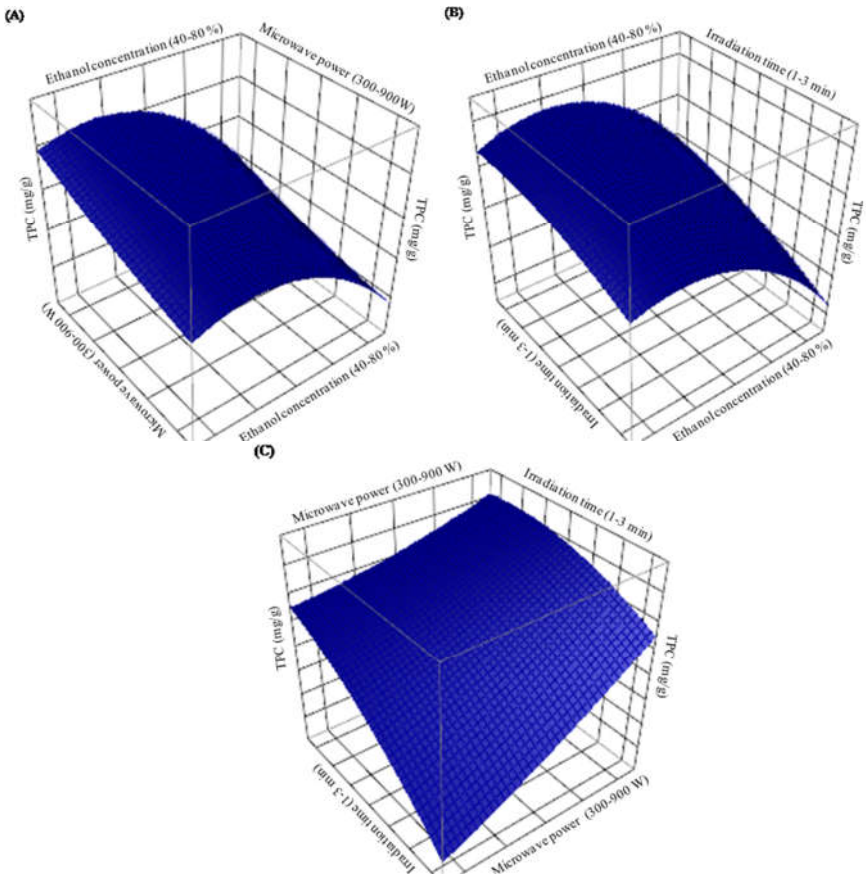

Figure 1. Response surface analysis for the total phenolic yield from *Phoenix dactylifera* L. by the microwave–assisted extraction with respect to ethanol's concentration and microwave power (a); ethanol's concentration and irradiation time (b) microwave power and irradiation time (c).

The effect of the microwave power and different levels of irradiation time on the extraction of TPC can be predicted from the surface plot as it is shown in Fig. 1c. It was confirmed from Fig. 1c that both of the independent variables had a strong effect on the extraction. The contour plot showed that the maximum extraction efficiency (4.00±0.06 mg GAE/g DW) was achieved under a 700 W microwave power and 2 min irradiation time. Increasing the microwave power from 300 to 700 W and the irradiation time from 1 to 2 min significantly increased the extraction rate of TPC from *Phoenix dactylifera* L. fruit. However, the further increase of the irradiation time from 2 to 3 min caused a decrease of the extraction yield.

Model fitting and statistical analysis

The response surface methodology (RSM) is an empirical modeling technique. It calculates the correlation between the actual and the forecast outcomes. To attain a suitable model so as to optimize the MAE (Ethanol's concentration, microwave power and irradiation time), the Box-Behnken design (BBD) was used (Table 2).

To understand the effect of the independent factors on the *Phoenix dactylifera* L. fruit's TPC yield and to determine the optimum solutions, the experimental design was followed. Table 2 presents the experimental data and the anticipated values. The yield of TPC ranged from 2.51±0.05 to 4.00±0.06 mg GAE/g DW.

The multiple regression analysis was applied to the experimental data, the response variable and the test variables were related to the following second-order polynomial equation according to the coded values:

$$Y(TPC) = 3.62 - 0.37X_1 + 0.24X_2 + 0.27X_3 - 0.01X_1X_2 + 0.07X_1X_3 - 0.10X_2X_3 - 0.32X_1^2 + 0.03X_2^2 - 0.09X_3^2$$

Where Y is the yield of TPC calculated by the regression model and X_1, X_2 and X_3 are the coded variables.

To avoid poor or disingenuous response surface results, the adequacy and fitness of the model were checked using the regression analysis and ANOVA (Sangamithra *et al.*, 2015). ANOVA subdivides the total variation of the results into smaller parts coupled with the sources of variation to test the hypotheses about the independent factors (Swamy *et al.*, 2014). Further, the Fisher's statistical test (*F*–test) analyzed the significance of each individual independent factor.

The ANOVA's results (Table 3) for the quadratic model showed that the polynomial model was statistically significant, as suggested by the high *F*-value (F=50.80) and the low *p*-value (p<0.0001). The *p* and *F* values are used to check the significance of each coefficient, while they also indicate the interaction strength between independent variables. The higher the *F*-value and the lower the *p*-value indicate the high significance of the corresponding coefficient.

The R^2 and the adjusted R^2 confirm the adequacy and suitability of the model. The high values of the determination coefficient (R^2=0.99) and the adjusted determination coefficient (R^2_{adj}=0.97) showed a high degree of correlation between the experimental and the predicted values of the TPC yield. The pure error was very low, indicating the good reproducibility of the obtained data. The model also showed that the lack of fit was statistically insignificant (p>0.05), which further confirmed the validity of the model.

At the same time, our results also demonstrated that the linear coefficients (X_1, X_2 and X_3), the quadratic coefficients (X_1^2 and X_2^2) and the interaction coefficients ($X_1 \times X_3$) have significant effects, with low *p*-values (p<0.05). The other coefficients (X_3^2, $X_1 \times X_2$ and $X_2 \times X_3$) were found to be insignificant (p>0.05). Moreover, the ethanol's concentration (X_1) was the most significant parameter that influenced the yield of TPC, followed by microwave power (X_2) and extraction time (X_3).

Table 3. Analysis of variance (ANOVA) for the experimental results obtained by using the microwave-assisted extraction.

	Sum of squares	***F*–value**	***p*–value**
Model	3.62	50.80	< 0.0001
X_1–Ethanol concentration	-0.37	-13.79	< 0.0001
X_2–Microwave power	0.24	9.17	0.0003
X_3–Irradiation time	0.27	10.00	0.0002
X_1X_2	-0.01	-0.26	0.8062
X_1X_3	0.07	1.92	0.1134
X_2X_3	-0.10	-2.56	0.0506
X_1^2	-0.32	-8.23	0.0004
X_2^2	0.03	0.74	0.4918
X_3^2	-0.09	-2.23	0.0763
Residual	0.03		
Lack of Fit	0.02	1,50	0.42
Pure Error	0.00		
R^2	0.99		
R^2_{adj}	0.97		
RMSE	0.07		

Investigating the model adequacy

Diagnostic plots ensure the model's adequacy and exhibit the correlation between the actual and the predicted results. The data points were close to the straight line, signifying a close agreement between the two data. It also suggests that the model is capable to select suitable operating conditions to extract the TPC from dates. Further, the normality assumption was verified by plotting a normal probability plot of the residuals. The data was found to lie near the straight line, so that it was concluded that the data was normally distributed.

Comparison between the extraction techniques

MAE is an innovative technique that has been presenting a special interest. Microwaves consist of a non-ionizing electromagnetic energy that is applied directly to the raw material. They transmit energy which penetrates into the biologic matrix and interacts with the polar molecules, mostly water, generating heat; the heat expands and disrupts the vegetal cell, favoring the extraction of intracellular phytochemical compounds. MAE is a technique frequently used to extract thermolabile compounds (Takeuchi *et al*., 2008). The UAE technique consists in using mechanic vibrations caused by sound waves with frequencies higher than 20 kHz. Sound waves are intrinsically different from electromagnetic waves, because the latter can propagate through the vacuum, while sound waves need a physical medium to propagate.

The mechanic vibrations cause expansion and compression cycles in the medium, creating bubbles which collapse and cause cavitation, instantly creating a high local pressure and intense local heating. These fast changes induce the disruption and thinning of the cell membranes, consequently increasing the mass transfer rate of

organic substances from the solid matrix to the solvent (Esclapez *et al*., 2011; Takeuchi *et al*., 2008). The conventional solid-liquid extraction can generate undesirable residues with products. In addition, the extract can suffer oxidative transformations during the solvent's removal step (Díaz-Reinoso *et al*., 2006).

Based on the optimum treatment parameter values (solvent concentration of 50%, power level of 700 W, and 2.42 min of extraction time) for maximizing phenolics, the best resulting MAE process was used to compare the total phenolic content. An amount of 2.82±0.05 and 2.27±0.04 mg GAE/g DW of phenolics were extracted when UAE (60 min) and maceration (60 min) extraction techniques were used. MAE, after 2.42 min of extraction, not only reduced the extraction time and the solvent's consumption but also gave a higher yield of phenolics compared to the time consuming UAE and classical-extraction technique which was 4.27±0.09 mg GAE/g DW.

Several scientific investigations report the MAE applicability for obtaining natural antioxidants, without generating undesirable residues (Li *et al*., 2011; Song *et al*., 2011; Sutivisedsak *et al*., 2010). In general, the antioxidant activity of the extracts obtained by MAE is higher than the activity of the extracts obtained by conventional techniques, because the microwave treatment does not cause any deterioration of the antioxidant properties of the extract (Li *et al*., 2012). MAE process is short, usually from 2 min to 40 min. This fact makes MAE an attractive technique, only for thermolabile compounds, although long extraction times can result in the degradation and consequent antioxidant capacity loss (Li *et al*., 2011).

The comparison of the total phenolic contents to those reported in the literature showed that they very similar to some studies and in contrast to others. Indeed, the study of Hamad *et al*. (2015) performed on the extracts of twelve cultivars of *Phoenix dactylifera* L. from Saudi Arabia showed that the total phenols content ranged from 10.4 to 22.1 mg GAE/100g DW. In addition, a study by Biglari *et al*. (2008) showed that the total polyphenols content of dates from Iran ranged from 2.89 to 6.64 mg GAE/100g DW, with the exception of the Kharak variety, whose total polyphenols content was 141.35 mg GAE/100g DW. The study done by Al-Farsi *et al*. (2007) showed that the total phenols content of the aqueous extracts of three date varieties (Mabseeli, Um-sellah and Shahal) from Omania was between 172 and 246 mg GAE/100 g DW. Kchaou *et al*. (2013) tested the extracts of the date varieties of Tunisia, they found that the total phenolic content varied between 240.38 to 505.49 mg GAE/100 g DW. Taking into account the obtained results, the MAE extract was selected to evaluate the antioxidant activity of dates.

The antioxidant potential of the phenolic extract in terms of the DPPH radical scavenging activity may be due to the existence of hydroxyls in the phenolic extract's molecule, which could be as an electron donator and transfer electron to the DPPH free radical (Krings and Berger, 2001). The percentage of the free radical DPPH inhibition was 58.72±0.29 %. The reducing power of the phenolic extract was 1.88±0.09 mg AscAE /mL. The reducing capacity of a compound may serve as an indicator of the potential antioxidant activity for date. Guo *et al*. (2003) reported that the date has the second highest antioxidant activity among 28 fruits

commonly consumed in China. On the other hand, it should be recalled that the phenolic profile of dates has revealed that the main phenolic compounds present in dates are cinnamic, ferulic and sinapic acids and their derivatives, as well as flavonoids (Mansouri *et al*., 2005). According to Cuvelier *et al*. (1992), caffeic, sinapic and ferulic acids are more active than protocatechic, syringic, vanillic and p-hydroxybenzoic acids.

RP-HPLC-PAD-ESI-MS/MS analysis

The RP-HPLC-PAD-ESI-MS/MS screening of the dates' extract obtained by microwave-assisted extraction (MAE), was illustrated in the HPLC-UV (254 nm) chromatogram (Figure 2), which exhibited numerous peaks, the peaks being numbered following the elution order (compounds 1-44).

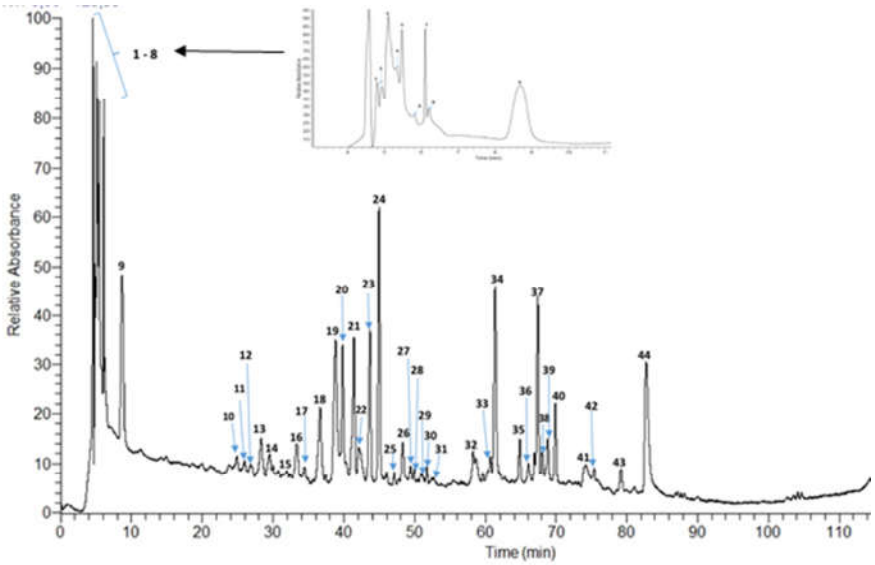

Figure 2. HPLC chromatogram of the date's extract recorded at 254 nm (inserted figure represents the magnification of the first 11 minutes of HPLC chromatogram).

Tables 4, 5, 6, 7, 8 and 9 show different compounds (sugars, organic acids, nucleosides, carotenoids, proanthocyanidins, lignans, flavonols, flavones, saponin and sterol glucosides) that grouped according to their structural similarity and following their elution order, as well as the Rt, MS data, UV max. and MS^n fragmentation pattern. In the chromatographic profile, there was a large peak (3) with an UV spectrum corresponding to gluconic acid disaccharide.

Sugars and organic acids

The first compounds to elute from the C18 column, as it could be predicted, were the sugars and organic acids as they were detected only in the negative ionization mode (Table 4).

Table 4. RP-HPLC-PAD-ESI-MS/MS analysis of sugars and organic acids.

Peak No	RT (min)	λ Max (nm)	$[M-H]^-$	Fragments $[M-H]^-$ found	$[M+H]^+$	Fragments $[M+H]^+$ found	Proposed structure
SUGARS AND ORGANIC ACIDS							
1	4.80	226, 265	387/ 341	341 (100)→ 179 (<5)			Dihexoside
2	4.92	226, 263	537	537 → 195 (100), 177 (10)	/	/	Gluconic acid disaccharide adduct
3	5.11	226, 264	537	537 → 195 (100), 177 (10)	/	/	Gluconic acid disaccharide adduct
4	5.30	226, 264	537	537 → 195 (100), 177 (10)	/	/	Gluconic acid disaccharide adduct
5	5.46	226, 259	537	537 → 195 (100), 177 (10)	/	/	Gluconic acid disaccharide adduct
6	5.76	223, 262	133	133 → 115 (100), 113 (10), 87 (<5), 71 (<5)	/	/	Malic acid
7	6.10	230, 259	191	191 →111(100), 173 (30)	/	/	Citric acid
8	6.19	226, 256	191	191 →111(100), 173 (30)	/	/	Citric acid

Peak 1 has been identified as a disaccharide with a m/z at 341, which breaks down to the 179 [M-H-162] fragment ions due to the loss of a hexosyl moiety (Sokeng *et al*., 2019). Peaks 2, 3, 4 and 5, instead all have the m/z of 537$[M-H-342]^-$ whose fragments lose a disaccharide to give m/z 195 which corresponds to gluconic acid. Peak 6 has been identified as malic acid at a m/z 133, it breaks to its basic fragment at a m/z 115$[M-H-H20]^-$ due to the loss of a water molecule.

Peaks 7 and 8 have been identified as citric acid at a m/z of 191, whose fragments give different daughter ions at the m/z 111$[M-H-80]^-$ and 173$[M-H-H_2O]^-$ which are characteristic of its fragmentation pattern. The identification of the sugars and the organic acids was confirmed by comparison to the reference standards.

Nucleoside and carotenoids

Nucleoside and carotenoids were identified only in the positive ionization mode as described in Table 5.

Table 5. RP-HPLC-PAD-ESI-MS/MS analysis of nucleoside and carotenoids.

Peak No	RT (min)	λ Max (nm)	$[M-H]^-$	Fragments $[M-H]^-$ found	$[M+H]^+$	Fragments $[M+H]^+$ found	Proposed structure
NUCLEOSIDE AND CAROTENOIDS							
9	8.71	220, 297	/	/	268	268→136(100), 106(<5)	Adenosine
20	35.49	229, 282, 322	/	/	541	541→379 (100), 361 (40), 306 (<5)	10-apo-β-caroten-10-ol hexoside
21	36.66	202, 232, 326	/	/	571	571→409 (100), 481 (30), 443 (10), 391 (10)	Hexoside of 3-hydroxy-α-apo-10-carotenoic acid
23	38.34	233, 286, 326	/	/	703	703→541 (100), 523 (20), 592(10) 541→379 (100), 361 (40), 331 (10), 460 (<5), 436(<5)	10-apo-β-caroten-10'ol dihexoside
24	39.07	232, 281, 324	/	/	733	733→571(100) 571→409 (100), 481 (30), 443 (10), 391(10)	Disaccharide of 3-hydroxy-α-apo-10-carotenoic acid

Peak 9 was identified as adenosine only in the positive ionization mode at m/z 268, this molecular ion breaks down to m/z 136$[M+H-132]^+$ due to the loss of a ribose moiety (Liu *et al*., 2008). Peak 20 at m/z was tentatively identified as 10-apo-β-caroten-10-ol hexoside, having a molecular ion at m/z 541 which breaks to 10-apo-β-caroten-10-ol, its principal fragment at m/z 379 $[M-H-162]^-$ due to the loss of a hexosyl moiety, 10-apo-β-caroten-10-ol then fragments at its turn to m/z 361 $[M+H-H_2O]^+$ and 306 $[M+2H-56]^+$ which are characteristic of this molecule (De Rosso and Mercadante, 2007). As previously seen, peak 21 at m/z 571 corresponds to 3-hydroxy-α-apo-10-carotenoic acid hexoside. Peak 23 instead at m/z 703 has been tentatively identified as 10-apo-β-caroten-10-ol dihexoside, in fact, it suddenly breaks down to 10-apo-β-caroten-10-ol hexoside at m/z 541$[M+H-162]^+$ by the loss of a hexosyl moiety, the resulting 10-apo-β-caroten-10-ol hexoside then breaks down at its turn as previously described above.

Peak 24 at m/z 733 has been identified as a disaccharide of 3-hydroxy-α-apo-10-carotenoic acid, it fragments losing a hexosyl moiety to give its monosaccharidic form at m/z 571 which is 3-hydroxy-α-apo-10-carotenoic acid hexoside, this later then losses at its turn another hexose moiety giving rise to the aglicone3-hydroxy-

α-apo-10-carotenoic acid at m/z 409 which then fragments as reported on Table 5 (Floss *et al.*, 2008).

Proanthocyanidins and lignans

Like carotenoids, in this chemical characterization, proanthocyanidins were identified only in the positive ionization mode (Table 6).

Table 6. RP-HPLC-PAD-ESI-MS/MS analysis of proanthocyanidins and lignans.

Peak No	RT (min)	λ Max (nm)	$[M-H]^-$	Fragments $[M-H]^-$ found	$[M+H]^+$	Fragments $[M+H]^+$ found	Proposed structure
PROANTHOCYANIDINS AND LIGNANS							
13	26.20	228, 277	/	/	579	427 (100), 409 (60), 291 (50), 488 (30)	Procyanidin dimer
17	34	227, 278, 305	/	/	867	577(100), 409(70), 427(60), 722(60), 715(40), 806(30), 699(30), 741(10), 623(<5), 553(30), 535(30), 451(30), 323(20)	Procyanidin trimer
19	26.20	228, 277	523	477 (100), 361(15)			Secoisolariciresinol-O-hexoside

Peak 13 at m/z 579 has been identified as procyanidin dimer. However, peak 17 at m/z 867 was tentative identified as procyanidin trimer, it breaks down to its common fragments 577[M+H]- (Table 6). Peak 19 at m/z 523 corresponds to secoisolariciresinol-*O*-hexoside. These results are in accordance to the mass spectrum of the reference standard and to the study of Sokeng *et al.* (2019).

Flavonols and flavones

Regarding flavonols and flavones, they were identified in the positive as well as in the negative ionization modes (Table 7). Peak 18 at m/z 449 has been identified as dihydrokaempferol-hexoside in the negative ionization mode. As seen from Table 7, this molecular ion fragments to its basic fragment ions at m/z 287$[M-H-162]^-$

which is dihydrokaempferol due to the loss of the sugar moiety, 269[M-H-180]$^-$ due to the loss of the sugar and a water molecule and 431[M-H-H_2O]$^-$ due to the loss of a water molecule (Mena *et al*., 2012).

Peak 23 has been identified in the positive ionization mode as 6,8-C-diglucosyl apigenin, it fragmented as reported in Table 7. Peaks 32 and 33 have been identified as kaempferol-3-O-rutinoside with the molecular ion at m/z 595 in the positive ionization which fragments to ions at m/z 449[M+H-146]$^+$ and 287[M+H-308]$^+$ due to the loss of rhamnosyl and rutinosyl respectively (Bamawa *et al*., 2018).

Peak 34 with molecular ions at the m/z 609 and 611, detected in negative and positive ionization modes respectively, was identified as rutin, due to its fragmentation pattern which includes fragment ions typical of quercetin derivatives. Due to the loss of the disaccharide rutinose (fragment ion at m/z 302), it corresponded to [M-H-α-L-rhamnopyranosyl-(1→6)-β-D-gluco-pyranose]-. Peaks 35 and 37 with the respective m/z of 607 and 609 in the negative and positive ionization modes, have been identified as chrysoeriol-7-O-neohesperidoside, in both modes, it fragments losing the sugar moiety neohesperidoside (made of rhamnose and glucose) to form the fragment ion chrysoriol at m/z 299 and 301 in the negative and positive ionization modes respectively. Instead, peak 36 at m/z 463 has been identified only in the positive ionization mode, it has been tentatively identified as chrysoeriol hexoside, it fragments losing the sugar to form the fragment ion chrysoriol at m/z 301[M+H-162]$^+$.

A comparison to the reference standard (rutin) confirmed this result. Peak 39 at m/z 447 has been identified only in the negative ionization mode as isorhamnetin-3-O-pentoside, it breaks down to its principal fragment at m/z 314[M-2H-132]$^-$ due to the loss of the sugar moiety (Schieber *et al*., 2002). Peak 40 at m/z 647 has been identified in the positive ionization mode as isorhamnetin-3-O-rutinoside, it fragments generating the ions at m/z 501[M+H+Na-146]$^+$ and 331[M+H+Na-316]$^+$ by losing a rhamnosyl unit and isorhamnetinaglicone respectively (De Bertoldi *et al*., 2012).

Peaks 41 and 42 at m/z 543 have been identified in the negative ionization mode as quercetin-hexosyl-sulphate, this molecular ion breaks to give its principal fragment at m/z 301[M-H-242]$^-$ due to the loss of a hexosyl-sulphate moiety. Peak 43 at m/z 519 has been identified in the negative ionization mode as isorhamnetin acetyl-hexoside, it fragments to its basic ions 314[M-2H-204]$^-$, 315[M-H-204]$^-$ due to the loss of the acetyl-hexoside, it also gives the fragment at m/z 357[M-H-162]$^-$ due to the loss of the sugar moiety, others fragments typical of this molecule were reported in Table 7 (Sobeh *et al*., 2016).

Peak 44 has been identified as chrysoeriolhexosyl sulphate, it has an m/z of 541 in the negative ionization mode which fragments tochrysoeriol at m/z 299[M-H-hexosylsulphate]$^-$ while in the positive mode it has an m/z of 543 which principally fragments tochrysoeriol at m/z 301[M+H-hexosyl sulphate]$^+$ and chrysoeriol hexoside at m/z 463[M+H-SO4]$^+$.

Table 7. RP-HPLC-PAD-ESI-MS/MS analysis of flavonols and flavones.

Peak No	RT (min)	λ Max (nm)	$[M-H]^-$	Fragments $[M-H]^-$ found	$[M+H]^+$	Fragments $[M+H]^+$ found	Proposed structure
				FLAVONOLS AND FLAVONES			
18	37.07	218, 274, 305	449	287(100), 269(40), 431(30), 243 (10)	/	/	Dihydrokaempferol hexoside
23	38.34	233, 286, 326	/	/	595	577(100), 457(50), 475(20), 559(40), 528(30)	6,8-C-diglucosylapigenin
32	58,2	233, 268, 333	/	/	595	449(100), 287(30)	Kaempferol-3-O-rutinoside
33	61.00	234, 268, 324	/	/	595	287(100), 433(30)	Kaempferol- 3-O-rutinoside
34	61.31	255, 352, 377	609	301(100), 343(15), 271(15), 179(<5)	611	515(100),465(60), 303(60), 345(50), 417(40), 382(30), 494(30), 543(30)	Rutin
35	64.20	234, 268, 332	/	/	609	463 (100), 301(15)	chrysoeriol-7-O-neohesperid- oside
36	56.50	234, 272, 319	/	/	463	301(100)	Chrysoeriol hexoside
37	67.45	234, 268, 333	653	299(100), 284(50)	609	301(100), 463(30)	chrysoeriol-7-O-neohesperi-doside
39	68.99	253, 267, 344	447	314(100), 357(20), 285(10), 179(<5), 152(<5), 399(<5), 329(<5)	/	/	Isorhamnetin-3-O-pentoside
40	69.94	255, 268, 346, 377	/	/	647	501(90),331(100), 619(10), 489(10), 338(10)	Isorhamnetin-3-O-rutinoside
41	74.19	247, 268, 338, 377	543	301(100), 229(10)	/	/	Quercetin hexosyl sulfate
42	74.46	247, 268, 338, 377	543	301(100), 229(10)	/	/	Quercetin hexosyl sulfate
43	79	215, 311, 376	519	314(100), 315(30), 357(15), 257(40), 491(20), 459(10), 223(10)	/	/	Isorhamnetinac-etyl hexoside
44	82.74	251, 347	541	299(100), 24(30)	543	301(100), 463(30), 504(10)	Chrysoeriol hexosyl sulfate

Saponin and sterol glucosides

From the HPLC-PDA-ESI-MS/MS analysis, eleuthoroside E formate adduct and disaccharide formate adduct have been identified (Table 8).

Table 8. RP-HPLC-PAD-ESI-MS/MS analysis of saponin and sterol glucosides.

Peak No	RT (min)	λ Max (nm)	$[M-H]^-$	Fragments $[M-H]^-$ found	$[M+H]^+$	Fragments $[M+H]^+$ found	Proposed structure
SAPONIN AND STEROL GLUCOSIDES							
25	47.02	202, 238, 301, 378	1111	949(100), 787(100) 741(80), 579(100), 353(30), 625(20) 579(100) → 417(100)	/	/	Eleutheroside E disacchardide formate adduct
26	48.29	202, 240, 281, 318, 377	787	741(100) → 579(100), 353 (65), 579(100) → 417(100)	/	/	Eleutheroside E formate adduct

Peak 25 at the m/z 1111 has been identified as eleuthoroside E disaccharide formate adduct, it fragments losing the disaccharide and the formic acid to give the fragment ion eleuthoroside at the m/z 741 which then fragments as previously described (Xiao *et al.*, 2018). In fact, peak 26 at the m/z 787 in the negative ionization mode breaks down releasing formic acid to give the fragment ion at the m/z 741 which corresponds to eleuthoroside E. Eleuthoroside E then breaks in turn to the give the fragment ions 579 and 417 due to the loss of one and two glucose moieties, respectively.

Unknown compounds

Peaks 10, 11, 12, 14, 15, 16, 20, 22, 27, 28, 29, 30 and 31 were not identified either in the negative or the positive ionization modes (Table 9).

Numerous studies have focused on the identification of phenolic compounds in the dates' extracts by HPLC analysis. Indeed, the study of Saleh *et al.* (2011) assessed the rutin, caffeic acid and catechin contents of the methanolic extracts of *Phoenix dactylifera* L. cultivars grown in Saudi Arabia. Boudries *et al.* (2007) noted that the chromatographic analyses of the Algerian varieties of *Phoenix dactylifera* L. revealed the dominance of the carotenoid pigments represented by lutein and β-carotene.

Dhaouadi *et al*. (2010) quantified the phenolic compounds, gallic acid, vanillic acid, cinnamic acid, 3,4-dicaffeoylquinic acid, 5-O-caffeoylshikimic, caffeoyl-sinapylquinic acid and caffeic acid, coumaric acid, in the aqueous extract of the Deglet-Nour variety grown in Tunisia. Similarly, the phenolic profile of the hydro-acetonic extracts of three Tunisian date cultivars established by Kchaou *et al.* (2016) where gallic acid, vanillic acid, caffeic acid, syringic acid, coumaric acid, ferulic acid, sinapic acid and rutin have been identified. The analysis of the

phenolic compounds of four varieties of Omani date by HPLC revealed the presence of gallic acid, coumaric acid, caffeic acid, vanillic acid and syringic acid (Al Harthi *et al.*, 2015). The chromatographic separation of aqueous and ethanolic extracts from Egyptian date cultivars revealed the presence of gallic acid, tannic acid and ferulic acid, respectively. In contrast, the study reported the absence of cinnamic acid (El Sohaimy *et al.*, 2015). The differences between our results and those in the literature can be attributed to the geographical origin of the fruits (Al Harthi *et al.*, 2015), the extraction method. In addition, solvent could be at the origin of the variations in the phenolic compounds of the date extract (Kchaou *et al.*, 2013; Shahdadi *et al.*, 2015; Amiour and Hambaba, 2016; Mrabet *et al.*, 2016).

Table 9. RP-HPLC-PAD-ESI-MS/MS analysis of unknown compounds.

Peak No	RT (min)	λ Max (nm)	$[M-H]^-$	Fragments $[M-H]^-$ found	$[M+H]^+$	Fragments $[M+H]^+$found	Proposed structure
UNKNOWN COMPOUNDS							
10, 11, 12, 15, 20, 22, 27, 28, 29, 30, 31	/	/	/	/	/	/	/
14	28	228, 278, 309	/	/	449	335 (100), 317(80), 274(30), 214(10), 143 (<5)	/
16	34	224, 278	/	/	/	317(100),335(50), 336(20), 4 03(10), 463(10), 241(10), 189(<59	/

Conclusions

The use of the common dates with low market value for the extraction of beneficial phytonutrients such as phenolic antioxidants not only provides health benefits but also adds values to the waste. The aim of this work was to characterize the phytochemical composition of the phenolic extract after the optimization of the microwave-assisted extraction using the response surface methodology that was chosen as the best method of extracting the phenolic compounds compared to ultrasound-assisted and conventional extractions, and evaluating the *in vitro* antioxidant activity of the extract in question. The antioxidant activity of the optimized extract was evaluated and compared using the following methods: the DPPH and the reducing power assays with the characterization of the dates extract metabolic profile by RP-HPLC-PAD-ESI-MS/MS. This study scientifically validated the use of dates in traditional medicine and revealed their interest in the context of exploitation in the field of biotechnology. The future work will focus on the optimization and large scale extraction of the selected phytonutrients from dates and their use as food additives.

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References

Abbès, F., Kchaou, W., Blecker, C., Ongena, M., Lognay, G., Attia, H., Besbes, S. 2013. Effect of processing conditions on phenolic compounds and antioxidant properties of date syrup. *Industrial Crops and Products*, **44**, 634-642.

Abu-Reidah, I.M., Gil-Izquierdo, Á., Medina, S., Ferreres, F. 2017. Phenolic composition profiling of different edible parts and by-products of date palm (*Phoenix dactylifera* L.) by using HPLC-DAD-ESI/MSn. *Food Research International*, **100**, 494-500.

Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., Al-Rawahy, F. 2007. Compositional and functional characteristics of dates, syrups, and their by-products. *Food Chemistry,* **104**(3), 943-947.

Al Harthi, S., Mavazhe, A., Al Mahroqi, H., Khan, S. 2015. Quantification of phenolic compounds, evaluation of physicochemical properties and antioxidant activity of four date (*Phoenix dactylifera* L.) varieties of Oman. *Journal of Taibah University Medical Sciences,* **10**(3), 346-352.

Al-Turki, S., Shahba, M.A., Stushnoff, C. 2010. Diversity of antioxidant properties and phenolic content of date palm (*Phoenix dactylifera* L.) fruits as affected by cultivar and location. *J. Food Agric. Environ*, **8**(1), 253-260.

Amiour, S.D., Hambaba, L. 2016. Effect of pH, temperature and some chemicals on polyphenoloxidase and peroxidase activities in harvested Deglet Nour and Ghars dates. *Postharvest Biology and Technology,* **111**, 77-82.

Ballard, T.S., Mallikarjunan, P., Zhou, K., O'Keefe, S. 2010. Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins. *Food Chemistry*, **120**(4), 1185-1192.

Bamawa, C., Ndjele, L., Foma, F. 2016. Characterization of leaf phenolic compounds of *Sabicea johnstonii* by HPLC-MS^n. *Journal of Natural Products and Resources*, **2**(2), 86–89.

Besbes, S., Drira, L., Blecker, C., Deroanne, C., Attia, H. 2009. Adding value to hard date (*Phoenix dactylifera* L.): compositional, functional and sensory characteristics of date jam. *Food Chemistry*, **112**(2), 406-411.

Biglari, F., AlKarkhi, A.F., Easa, A.M. 2008. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. *Food Chemistry,* **107**(4), 1636-1641.

Boudries, H., Kefalas, P., Hornero-Méndez, D. 2007. Carotenoid composition of Algerian date varieties (*Phoenix dactylifera*) at different edible maturation stages. *Food Chemistry,* **101**(4), 1372-1377.

Camel, V. 2000. Microwave-assisted solvent extraction of environmental samples. *TrACTrends in Analytical Chemistry*, **19**(4), 229-248.

Chan, C.H., Yusoff, R., Ngoh, G.C. 2014. Optimization of microwave-assisted extraction based on absorbed microwave power and energy. *Chemical Engineering Science*, **111**, 41-47.

Chandrasekaran, M., Bahkali, A.H. 2013. Valorization of date palm (*Phoenix dactylifera*) fruit processing by-products and wastes using bioprocess technology–Review. *Saudi Journal of Biological Sciences*, **20**(2), 105-120.

Chemat, F., Abert-Vian, M., Fernandez, X. 2012. Microwave-assisted extraction of essential oils and aromas. In *Microwave-assisted extraction for bioactive compounds*, Springer, Boston, MA., 53-68.

Chibane, H., Benamara, S., Noui, Y., Djouab, A. 2007. Some physicochemical and morphological characterizations of three varieties of Algerian common dates. *European Journal of Scientific Research*, **18**(1), 134-140.

Chu, Y.F., Sun, J., Wu, X., Liu, R.H. 2002. Antioxidant and antiproliferative activities of common vegetables. J*ournal of Agricultural and Food Chemistry*, **50**(23), 6910-6916.

Cuvelier, M.E., Richard, H., Berset, C. 1992. Comparison of the antioxidative activity of some acid-phenols: structure-activity relationship. *Bioscience, Biotechnology, and Biochemistry*, **56**(2), 324-325.

Dai, J., Yaylayan, V.A., Raghavan, G.V., Parè, J.R. 1999. Extraction and colorimetric determination of azadirachtin-related limonoids in neem seed kernel. *Journal of Agricultural and Food Chemistry*, **47**(9), 3738-3742.

De Bertoldi, C., De Leo, M., Ercoli, L., Braca, A. 2012. Chemical profile of Festuca arundinacea extract showing allelochemical activity. *Chemoecology*, **22**(1), 13-21.

De Rosso, V.V., Mercadante, A.Z. 2007. Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. *Journal of Agricultural and Food Chemistry,* **55**(13), 5062-5072.

Dhaouadi, K., Raboudi, F., Estevan, C., Barrajón, E., Vilanova, E., Hamdaoui, M., Fattouch, S. 2010. Cell viability effects and antioxidant and antimicrobial activities of Tunisian date syrup (Rub El Tamer) polyphenolic extracts. *Journal of Agricultural and Food Chemistry,* **59**(1), 402-406.

Díaz-Reinoso, B., Moure, A., Domínguez, H., Parajó, J.C. 2006. Supercritical CO_2 extraction and purification of compounds with antioxidant activity. *Journal of Agricultural and Food Chemistry*, **54**(7), 2441-2469.

Djaoud, K., Arkoub-Djermoune, L., Remini, H., Sait, S., Tazarourte, M., Hadjal, S., Romero, A., Madani, K., Boulekbache-Makhlouf, L. 2019. Syrup from common date variety (*Phoenix dactylifera* L.): Optimization of sugars extraction and their quantification by High Performance Liquid Chromatography. *Current Nutrition & Food Science,***15**, 1-13.

Djaoud K., Boulekbache-Makhlouf L., Yahia M., Mansouri H., Mansouri N., Madani K., Romero A. 2020. Dairy dessert processing: Effect of sugar substitution by date syrup and powder on its quality characteristics. *Journal of Food Processing and Preservation*. e14414.

El Sohaimy, S., Abdelwahab, A., Brennan, C.S., Aboul-Enein, A. 2015. Phenolic content, antioxidant and antimicrobial activities of Egyptian date palm (*Phoenix dactylifera* L.) fruits. *Australian Journal of Basic and Applied Sciences*, **9**(1), 141-147.

Esclapez, M., García-Pérez, J.V., Mulet, A., Cárcel, J. 2011. Ultrasound-assisted extraction of natural products. *Food Engineering Reviews*, **3**(2), 108.

Floss, D.S., Schliemann, W., Schmidt, J., Strack, D., Walter, M.H. 2008. RNA interference-mediated repression of MtCCD1 in mycorrhizal roots of Medicago truncatula causes accumulation of C27 apocarotenoids, shedding light on the functional role of CCD1. *Plant Physiology*, **148**(3), 1267-1282.

Georgé, S., Brat, P., Alter, P., Amiot, M.J. 2005. Rapid determination of polyphenols and vitamin C in plant-derived products. *Journal of Agricultural and Food Chemistry*, **53**(5), 1370-1373.

Guo, C., Yang, J., Wei, J., Li, Y., Xu, J., Jiang, Y. 2003. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research*, **23**(12), 1719-1726.

Hamad, I., Abd Elgawad, H., Al Jaouni, S., Zinta, G., Asard, H., Hassan, S., Hegab, M., Hagagy, N., Selim, S. 2015. Metabolic analysis of various date palm fruit (*Phoenix dactylifera* L.) cultivars from Saudi Arabia to assess their nutritional quality. *Molecules*, **20**(8), 13620-13641.

Kchaou, W., Abbès, F., Blecker, C., Attia, H., Besbes, S. 2013. Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties (*Phoenix dactylifera* L.). *Industrial Crops and Products,* **45**, 262-269.

Kchaou, W., Abbès, F., Mansour, R.B., Blecker, C., Attia, H., Besbes, S. 2016. Phenolic profile, antibacterial and cytotoxic properties of second grade date extract from Tunisian cultivars (*Phoenix dactylifera* L.). *Food Chemistry,* **194**, 1048-1055.

Krings, U., Berger, R. 2001. Antioxidant activity of some roasted foods. *Food Chemistry*, **72**(2), 223-229.

Li, H., Deng, Z., Wu, T., Liu, R., Loewen, S., Tsao, R. 2012. Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. *Food Chemistry*, **130**(4), 928-936.

Li, Y., Skouroumounis, G.K., Elsey, G.M., Taylor, D.K. 2011. Microwave-assistance provides very rapid and efficient extraction of grape seed polyphenols. *Food Chemistry*, **129**(2), 570-576.

Liu, R., Ye, Y., Qiang, L., Liao, X., Zhao, Y. 2008. The fragmentation pathway of the nucleosides under the electrospray ionization multi-stage mass spectrometry. *Life Science Journal*, **5**, 37-40.

Mansouri, A., Embarek, G., Kokkalou, E., Kefalas, P. 2005. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). *Food Chemistry*, **89**(3), 411-420.

Mena, P., Calani, L., Dall'Asta, C., Galaverna, G., García-Viguera, C., Bruni, R., Crozier, A., Del Rio, D. 2012. Rapid and comprehensive evaluation of (poly) phenolic compounds in pomegranate (*Punica granatum* L.) juice by UHPLC-MSn. *Molecules*, **17**(12), 14821-14840.

Milardović, S., Iveković, D., Grabarić, B.S. 2006. A novel amperometric method for antioxidant activity determination using DPPH free radical. *Bioelectrochemistry*, **68**(2), 175-180.

Moraes, M.N., Zabot, G.L., Prado, J.M., Meireles, M.A.A. 2013. Obtaining antioxidants from botanic matrices applying novel extraction techniques. *Food and Public Health*, **3**(4), 195-214.

Mrabet, A., Jiménez-Araujo, A., Fernández-Bolaños, J., Rubio-Senent, F., Lama-Muñoz, A., Sindic, M., Rodríguez-Gutiérrez, G. 2016. Antioxidant phenolic extracts obtained from secondary Tunisian date varieties (*Phoenix dactylifera* L.) by hydrothermal treatments. *Food chemistry*, **196**, 917-924.

Oyaizu, M. 1986. Studies on products of browning reaction. *The Japanese Journal of Nutrition and Dietetics*, **44**(6), 307-315.

Saleh, E.A., Tawfik, M.S., Abu-Tarboush, H.M. 2011. Phenolic contents and antioxidant activity of various date palm (*Phoenix dactylifera* L.) fruits from Saudi Arabia. *Food and Nutrition Sciences,* **2**(10), 1134.

Sangamithra, A., Sivakumar, V., Kannan, K., John, S.G. 2015. Foam-mat drying of muskmelon. *International Journal of Food Engineering*, **11**(1), 127-137.

Schieber, A., Keller, P., Streker, P., Klaiber, I., Carle, R. 2002. Detection of isorhamnetin glycosides in extracts of apples (*Malus domestica* cv."Brettacher") by HPLC-PDA and HPLC-APCI-MS/MS. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, **13**(2), 87-94.

Setyaningsih, W., Saputro, I., Palma, M., Barroso, C. 2015. Optimisation and validation of the microwave-assisted extraction of phenolic compounds from rice grains. *Food Chemistry,* **169**, 141-149.

Shahdadi, F., Mirzaei, H., Garmakhany, A.D. 2015. Study of phenolic compound and antioxidant activity of date fruit as a function of ripening stages and drying process. *Journal of Food Science and Technology,* **52**(3), 1814-1819.

Sobeh, M., ElHawary, E., Peixoto, H., Labib, R.M., Handoussa, H., Swilam, N., El-Khatib, A.H., Sharapov, F., Mohamed, T., Krstin, S. 2016. Identification of phenolic secondary metabolites from Schotia brachypetala Sond. (*Fabaceae*) and demonstration of their antioxidant activities in Caenorhabditis elegans. *Peer J*, **4**, e2404.

Sokeng, A.J.T., Sobolev, A.P., Di Lorenzo, A., Xiao, J., Mannina, L., Capitani, D., Daglia, M. 2019. Metabolite characterization of powdered fruits and leaves from *Adansonia digitata* L.(Baobab): A multi-methodological approach. *Food Chemistry*, 272, 93-108.

Song, J., Li, D., Liu, C., Zhang, Y. 2011. Optimized microwave-assisted extraction of total phenolics (TP) from *Ipomoea batatas* leaves and its antioxidant activity. Innovative *Food Science & Emerging Technologies*, **12**(3), 282-287.

Sutivisedsak, N., Cheng, H., Willett, J., Lesch, W., Tangsrud, R., Biswas, A. 2010. Microwave-assisted extraction of phenolics from bean (*Phaseolus vulgaris* L.). *Food research international*, **43**(2), 516-519.

Swamy, G.J., Sangamithra, A., Chandrasekar, V. 2014. Response surface modeling and process optimization of aqueous extraction of natural pigments from *Beta vulgaris* using Box–Behnken design of experiments. *Dyes and Pigments*, **111**, 64-74.

Takeuchi, T.M., Pereira, C.G., Braga, M.E., Maróstica Jr, M.R., Leal, P.F., Meireles, M.A.A. 2008. Low-pressure solvent extraction (solid–liquid extraction, microwave assisted, and ultrasound assisted) from condimentary plants. In *Extracting Bioactive Compounds for Food Products*. *CRC Press*, 150-231.

Venkatesh, M., Raghavan, G. 2004. An overview of microwave processing and dielectric properties of agri-food materials. *Biosystems Engineering*, **88**(1), 1-18.

Xiao, Y., Liu, L., Bian, J., Yan, C., Ye, L., Zhao, M., Huang, Q., Wang, W., Liang, K., Shi, Z. 2018. Identification of multiple constituents in shuganjieyu capsule and rat plasma after oral administration by ultra-performance liquid chromatography coupled with electrospray ionization and ion trap mass spectrometry. *Acta Chromatographica*, **30**(2), 95-102.

Abstract

In Algeria, important quantities of dates of low commercial value (*Phoenix dactylifera* L.) are generated in each campaign, this dates can be valorized on syrup, which is a likely sweetener ingredient to be used in formulating food products to progress their nutritional properties. Therefore, the objectives of this work were, firstly, to evaluate the morphological and physicochemical characteristics of Degla-Beida date variety, then to determine the phytocomposition of the optimized phenolic extract obtained by microwave-assisted extraction (MAE), compared to the ultrasound-assisted and conventional extractions (UAE and CE), and to test its antioxidant activity *in vitro*. Secondary, to prepare date syrups and to evaluate the effect of conventional (CE) and alternative (MAE and UAE) processes on its total sugar content (TSC). Then to analyse their individual sugars using HPLC-RID. Finely, a new dairy desserts were formulated by the substitution of sucrose with date syrup (SDD), date powder (PDD) and diffrent concentrations of date syrup and powder (DS/DP) (MDD) using D-Optimal mixture design. Maximum total phenolic content (TPC) (4.27±0.09 mg GAE/g DW) was obtained. RP-HPLC-PDA-ESI-MS/MS analysis revealed the presence of 44 bioactive compounds. Maximum TSC recoveries under the optimized conditions for MAE, UAE and CE were: 233.80±1.90, 202.04±3.40 and 234.38±2.43 g/L, respectively. The optimized mixture proportions of the best dairy dessert formulation, containing date syrup (14%) and powder (2%) as a sugar substitute (0%), were determined. The results of this study allowed us to conclude that MAE is the best method that allows the extraction of TPC and TSC based on the lower extraction time and significant extraction efficiency. DS/DP can be used to develop nutritive foods with health benefits for consumers, the advantage becomes more economically sound when these food is developed from by-products.

Key words : Valorization, common dates, date syrup, dairy dessert, MAE.

Résumé

En Algérie, des quantités importantes de dattes de faible valeur marchande (*Phoenix dactylifera* L.) sont générées dans chaque campagne, ces dattes pourraient être valorisées en sirop, qui est un ingrédient édulcorant approprié à utiliser pour formuler des produits alimentaires afin de progresser leur propriétés nutritives. Ainsi, les objectifs de ce travail étaient, premièrement, d'évaluer les caractéristiques morphologiques et physico-chimiques de la variété de datte Degla-Beida, puis de déterminer la phytocomposition de l'extrait phénolique optimisé, obtenu par extraction-assistée par micro-ondes (EAM), comparer à l'éxtraction-assistée par ultrasons et l'éxtraction conventionnelle (EAU et EC), et à tester son activité antioxydante *in vitro*. Deuxièmement, de préparer des sirops de dattes et d'évaluer l'effet des procédés conventionnel (EC) et alternatif (EAM et EAU) sur le rendement en sucre totaux (TST). Ensuite, d'analyser les sucres individuels par HPLC-RID. Finalement, de nouvelles crèmes desserts ont été formulées par la substitution du sucre par le sirop de dattes (CDS), la poudre de dattes (CDP) et différente concentrations de sirop et de poudre de dattes (SD/PD) (CDM) en utilisant le plan de mélange D-Optimal. Une teneur maximale en CPT de 4,27±0,09 mg EAG/g MS a été obtenus. L'analyse RP-HPLC-PDA-ESI-MS/MS a révélée la présence de 44 composés bioactifs. Les teneurs maximales en sucres totaux dans les conditions optimales pour EAM, EAU et EC étaient: 233,80±1,90; 202,04±3,40 et 234,38±2,43 g/L. Les proportions optimales de la meilleure formulation de crème dessert, contenant du sirop (14%) et de la poudre de datte (2%) comme substituant de sucre (0%), ont été déterminées. Les résultats de cette étude nous ont permis de conclure que l'extraction-assistée par microonde est la meilleure méthode qui permet l'extraction des composés phénoliques et des sucres totaux en fonction du temps d'extraction réduit et du rendement important. Le sirop et la poudre de dattes peuvent être utilisés pour développer des aliments nutritifs avec des avantages pour la santé des consommateurs, l'avantage devient plus économique lorsque ces aliments sont élaborés à partir de sous-produits.

Mots clés : Valorisation, dattes communes, sirop de dattes, crèmes desserts, EAM.

ملخص

في الجزائر، يتم إنتاج كميات كبيرة من التمور ذات القيمة السوقية المنخفضة (*Phoenix dactylifera* L.) في كل حملة، ويمكن تقييم ثمار التمر هذه إلى شراب، وهو مكون تحلية مناسب لاستخدامه في صياغة المنتجات الغذائية من أجل تحسين خصائصهم الغذائية. وهكذا، كانت أهداف هذا العمل، أولاً، تقييم الخصائص المورفولوجية والفيزيوكيميائية للتمور الجافة دقلة البيضاء، ثم تحديد التركيب النباتي لمستخلص الفينول الأمثل الذي تم الحصول عليه عن طريق الاستخراج بمساعدة الميكروويف (MAE) بمقارنة بالاستخراج بمساعدة الموجات فوق الصوتية والاستخراج التقليدي (UAE و CE)، واختبار نشاطه المضاد للأكسدة في المختبر. ثانياً، لتحضير شراب التمر وتقييم تأثير العمليات التقليدية (CE) والبديلة (MAE و UAE) على إنتاج السكر الكلي (TSC). بعد ذلك، تم تحليل السكريات الفردية بواسطة HPLC-RID. أخيرًا، تمت صياغة كريمات الحلوى الجديدة عن طريق استبدال السكر بشراب التمر (SDD)، ومسحوق التمر (PDD) وتركيزات مختلفة من شراب ومسحوق التمر (MDD) (DS/DP) باستخدام خطة الخلطD-Optimal. تم الحصول على الحد الأقصى لمحتوى TPCالبالغ 4.27±0.09 مجم/جم. كشف تحليل RP-HPLC-PDA-ESI-MS/MS عن وجود 44 مركبًا نشطًا بيولوجيًا. كان الحد الأقصى لمحتويات السكر الإجمالية المتوقعة في ظل الظروف المثلى لـ MAE ، UAE وCE: 233.60±1.90 ، 202.04±3.40 و 234.38±2.43 جم/لتر على التوالي. فيما يتعلق بالجزء الثالث، تم تحديد النسب المثلى لأفضل تركيبة كريمة الحلويات المحتوية على شراب (14٪) ومسحوق التمر (2٪) كبديل للسكر (0٪) للحصول على الحس الحسي المطلوب. سمحت لنا نتائج هذه الدراسة باستنتاج أن الاستخلاص بمساعدة الميكروويف هو أفضل طريقة تسمح باستخراج الفينولات والسكريات الكلية وفقًا لوقت الاستخراج المنخفض والإنتاجية العالية. يمكن استخدام مسحوق وشراب التمر في ذلك لتطوير أغذية مغذية ذات فوائد صحية للمستهلكين، تصبح الفائدة أكثر اقتصادا عندما يتم تصنيع هذه الأطعمة من منتجات ثانوية.

الكلمات المفتاحية : التقييم، التمور الجافة، شراب التمر، كريمات الحلويات، MAE.